



Marine Chemical Monitoring

*Policies, Techniques
and Metrological Principles*

Philippe Quevauviller

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André Mariotti

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Contents

Preface	ix
Glossary	xv
Abbreviations	xxi
Chapter 1. Marine Monitoring: Historical Background, Regulatory Framework and Science–Policy Interactions	1
1.1. Introduction	1
1.2. International institutions	3
1.2.1. International Council for the Exploration of the Sea	5
1.2.2. United Nations Environment Programme	6
1.2.3. Intergovernmental Oceanographic Commission of UNESCO	10
1.2.4. European Union	11
1.3. International conventions/programs	12
1.3.1. UN Convention on the Law of the Sea	12
1.3.2. London Dumping Convention	13
1.3.3. OSPAR Conventions	13
1.3.4. Helsinki Convention	16
1.3.5. MARPOL	17
1.3.6. Mediterranean Sea: Barcelona Convention	18
1.3.7. Bonn Agreement	19

1.3.8. Arctic Ocean: Arctic Monitoring and Assessment Programme	19
1.3.9. North East Pacific Ocean	20
1.3.10. North West Atlantic Ocean	21
1.3.11. North Sea conferences	22
1.3.12. Other conventions	22
1.4. The EU marine strategy.	22
1.4.1. The notion of “good environmental status”.	24
1.4.2. Marine strategies of the Member States	25
1.4.3. Monitoring in the MSFD policy context	26
1.5. Science–policy interactions	29
1.5.1. Scientific foundation of environmental policies: where do we stand?	29
1.5.2. EU scientific framework in support of water and marine policies	31
1.5.3. Identification of research needs in the water policy sectors	33
1.5.4. Interactions with the scientific community	34
1.5.5. Science-based development of an integrated environmental policy	37
1.6. Conclusions	39
Chapter 2. Monitoring and Quality Assurance	41
2.1. Monitoring of what?	41
2.1.1. Selection of compartments	41
2.1.2. Selection of compounds	42
2.2. Quality of data	44
2.2.1. Introduction	44
2.2.2. Interlaboratory comparisons.	45
2.2.3. Guidelines	47
2.2.4. (Certified) reference materials	48
2.2.5. Laboratory performance studies	49
2.2.6. Example: monitoring of trace metals in seawater	51
2.3. Certified reference materials	53
2.3.1. Introduction	53
2.3.2. Production and use of reference materials	53
2.3.3. CRMs for trace elements in nutrients	62
2.3.4. CRMs for organic non-halogenated compounds	66
2.3.5. CRMs for organic halogenated compounds	68
2.3.6. Future needs of CRMs	71

Chapter 3. Types of Monitoring	73
3.1. Classical chemical marine monitoring	73
3.1.1. Introduction	73
3.1.2. The basis and purpose of marine monitoring	74
3.1.3. Some considerations around classical monitoring	77
3.1.4. Designing a sampling program	80
3.1.5. Sample collection and immediate handling	82
3.1.6. Sample storage (short- and long-term)	83
3.1.7. Laboratory analyses	86
3.1.8. The final assessment.	93
3.1.9. Conclusions	94
3.2. <i>In situ</i> methods	94
3.2.1. Introduction	94
3.2.2. <i>In situ</i> automatic analyzers	96
3.2.3. Passive sampling technologies	99
3.2.4. Spectroscopic methods	106
3.2.5. Electrochemical techniques	110
3.2.6. Sensors	113
3.2.7. Biological early warning systems	116
3.2.8. Future	119
3.3. Biomonitoring	121
3.3.1. Introduction	121
3.3.2. Analytical trends in chemical monitoring of marine biota	123
3.3.3. Main features of biota monitoring programs	128
3.3.4. Analytical methods	131
3.3.5. Integration of chemical and biological effect monitoring	136
3.4. Use of sediment in coastal monitoring	139
3.4.1. Introduction	139
3.4.2. Sediment monitoring in the WFD context	142
3.4.3. Chemical monitoring in estuaries for coastal management	142
Chapter 4. Analytical Methods	147
4.1. Trace elements	147
4.1.1. Introduction	147
4.1.2. Digestion methods	148
4.1.3. Preconcentration methods for seawater analysis	150

4.1.4. Atomic absorption and emission techniques	151
4.1.5. (Instrumental) neutron activation analysis	157
4.1.6. X-ray techniques	158
4.1.7. Electrochemical techniques	159
4.1.8. Conclusions	160
4.2. Chemical species.	161
4.2.1. Introduction	161
4.2.2. Labile/complexed fractionation of metal species	163
4.2.3. Inorganic chromium species	168
4.2.4. Inorganic and organic arsenic species	171
4.2.5. Inorganic and methylated mercury species	176
4.2.6. Butyltin and other organotin species.	181
4.3. Organic micropollutants	185
4.3.1. Introduction	185
4.3.2. Polychlorinated biphenyls	186
4.3.3. Polybrominated diphenyls ethers.	189
4.3.4. Emerging contaminants	191
4.3.5. Organohalogens in water	193
4.3.6. Polycyclic aromatic hydrocarbons	196
4.4. Nutrients.	197
4.4.1. Introduction	197
4.4.2. Nutrient monitoring.	198
4.4.3. Analytical methods	199
Chapter 5. Conclusions: Achieving Traceability in Marine Monitoring Measurements?	205
5.1. Metrology in marine chemistry: traceability principles of chemical measurements	205
5.1.1. Meaning of traceability for chemical measurements	206
5.1.2. Stated references	209
5.1.3. Case studies illustrating metrology in marine chemistry	220
5.1.4. Conclusions	229
5.2. Policy perspectives.	231
Bibliography	235
Index	283

Preface

When I was invited to write this book, my first reaction was to wonder about my legitimacy to embark on this new editorial venture since my active involvement in oceanography seems to me far from current practices. Upon reflection, I however figured out that I have been involved in different facets of analytical developments, some of them related to marine science and policies for about 30 years, and that I could hence give it a try. In the specific field of “metrology” – the science of measurements – we cannot reinvent the wheel and I agreed to contribute a book related to this topic, which would be based on previous editorial projects to which I had contributed as author or editor. Writing or updating the different chapters of this book reminded me about my career path regarding chemical oceanography, which mixes research, analytical practices and policy, and of all the scientists with whom I worked and who contributed to my scientific and personal network. This preface is a tribute to them.

I started my university studies in marine geology in the early 1980s at the University of Bordeaux under the direction of Prof. Michel Vigneault and other scientists who opened my interest in oceanography, in particular Jean-Claude Faugères and Jean-Marie Froidefond. I joined the geochemistry discipline thanks to Francis Grousset at the Institut de Géologie du Bassin d’Aquitaine (IGBA, Geological Institute of Aquitaine Basin) and got my initial experience in environmental chemistry dealing with research into the silica

and dissolved oxygen pathways in a lake environment [QUE 84] under the supervision of Jean-Marie Jouanneau. I was trained by Philippe Pédemay who introduced me to water sampling and titrimetry/colourimetry. This readily taught me all the care that is required to avoid sample contamination and precautions to be taken regarding data accuracy. During this period, I was also involved in monitoring campaigns of physicochemical parameters in estuarine waters in front of the Blayais nuclear plant, navigating from Bordeaux (Port de la Lune) to the Gironde Estuary mouth on the *Ebalia* oceanographic vessel.

My second step into marine science was during my “Portuguese time” in the years 1984–1987 with in the framework of a scientific cooperation between the University of Bordeaux and the Portuguese Environment State Secretary. I inherited a study of a fantastic playground, in the form of an estuary (Sado Estuary) and a coastal environment (Galé Coast) that had to be studied in depth for geomorphology, sedimentology and geochemistry, under the supervision of Jean-Marie Jouanneau, Claude Latouche and Teresa Pera leading to a Doctorate [QUE 87a] in May 1987. I worked closely with outstanding researchers in Lisbon, in particular Alverinho Dias (then at the Portuguese Geological Surveys), Teresa Vinhas (Marine Hydrographical Institute), Carlos Vale (Fisheries Institute) and Leopoldo Cortez (then at the Environment State Secretary), as well as Miguel Aguas with whom I was introduced to modeling of coastal sediment dynamics, which formed the basis of my first paper published in the international literature [QUE 87b].

The “metrological” side of my studies focused on major and trace element determinations in estuarine sediment and biota samples. I discovered the joy of sampling muddy sediments and oysters in the area of Setúbal, carrying samples in the boot of my car on overnight trips to Bordeaux for major and trace element determinations mainly by X-ray fluorescence (XRF). I was also introduced to graphite furnace atomic absorption spectrometry (GFAAS) by Gilbert Lavaux at the IGBA for Cd and Pb measurements in sediments and oyster tissues. The complex pollution pathways of the Sado Estuary engaged me in further research [QUE 89], in particular about mobile forms of

trace metals using sequential extraction approaches (in particular, the “Tessier scheme”) and speciation because of a collaboration with Prof. Michel Astruc (University of Pau) and his team. In his laboratory, I learnt to analyze sediment and biota samples for their butyltin contents under the supervision of Renault Lavigne, and took this opportunity to learn anodic stripping voltammetry and cathodic stripping voltammetry under the supervision of André Castetbon. I did some further work on sediment analyses, in particular particulate organic carbon, under the supervision of Henri Etcheber at the Netherlands Institute for Sea Research (NIOZ). I also met with Wim Salomons to discuss sequential extraction matters.

Speciation opened a new phase of my oceanographic career thanks to Olivier Donard, who recruited me to assist in the development of a hybrid generation GCAs system for the determination of organotin compounds. This was done under a contract by the Rijkswatersta (Dutch Ministry of Water Works) to monitor organotin compounds in Dutch waters, the results of which would result in a policy leading to the banning of tributyltin antifouling paints. The policy side was managed by Remi Laane. With Olivier Donard, we were in charge of organizing two monitoring campaigns in estuaries, lakes and coastal waters of the Netherlands, and I took care of hundreds of samples of sediment, suspended matters biota and water in partnership with Rob Ritsema. This experience brought me into marine monitoring work covering the whole “measurement chain” from sampling to laboratory, in addition with a practical objective related to policy decision-making. It also allowed me to gain a second PhD (in environmental chemistry this time) in 1991 [QUE 91].

This international experience allowed me to join a European Commission research program, the so-called “Community Bureau of Reference” (BOR), thanks to meeting Ben Griepink at a conference in Lisbon (country of Michel Astruc). In this program, I was in charge of organizing interlaboratory studies and reference material certification campaigns in the speciation area [QUE 98], which allowed me to work with outstanding chemists, including Les Ebdon, Peter Craig, Rita Cornelis, Freddy Adams, Roberto Morabito, and Carmen Camara, to name a few. One of the BCR projects under my

responsibility, which was started at the request of OSPARCOM and the NSTF, concerned the need to develop a proficiency testing scheme for marine monitoring in order to improve the quality and comparability of measurement data. This formed the basis for the development of the QUASIMEME project [WEC 93] under the chairmanship of David Wells, and many other high-level marine scientists involved in the International Council for the Exploration of the Sea. At this time, I reflected upon the basic metrology principles that are behind environmental monitoring, including marine measurements, and was encouraged to return to research and development, which led me to undertake a HDR diploma in 1999 at the University of Pau [QUE 99a]. The plan to return to a purely scientific career did not concretize, and I decided to shift to policy development instead.

I joined the Environment Directorate-General of the European Commission in 2002. I have been in charge of developing a Directive on Groundwater Protection, a “daughter” directive of the Water Framework Directive (WFD), under the supervision of Patrick Murphy. In doing this, I did not leave the marine sector since the WFD has a coastal component, and I participated in discussions prior to the adoption of the Marine Strategy Directive. Using my quality assurance/quality control (QA/QC) experience, I was able to convince experts within a European group on chemical monitoring activity, which I co-shared with Mario Carere at that time, that a directive dealing with quality principles for chemical monitoring measurements under the WFD would be required. This led to the adoption of the Commission Directive 2009/90/EC, pursuant to Directive 2000/60/EC of the European Parliament and of the Council, laying down technical specifications for chemical analysis and monitoring of water status in 2009 [COM 09].

The sum of experiences gathered over the years allowed me to enter into the editorial world in the early 2000s, e.g. as contributing editor to *Trends in Analytical Chemistry* and as a member of editorial boards on journals, the edition and authoring of several books, and the coordination of a book series on “Water Quality Measurements”, which is composed of 10 books. One of the volumes falls directly

within the scope of the present book [QUE 11b] and much of its substance is hence related to this former publication, with appropriate references.

I was also keen to transmit my combined knowledge of laboratory experience, research and policy developments. Besides my work at the European Commission, I have been invited by André Van der Beken to join his group as associate professor at the Department of Hydrology and Hydrological Engineering at Vrije Universiteit Brussels to teach in an International Masters Programme on “Integrated Water Resource Management”. One of the aspects naturally concerns measurements and metrology principles.

This book consists of five chapters. Chapter 1 provides an insight into historical background, the regulatory framework and science–policy interactions, Chapter 2 deals with monitoring and related QA/QC, Chapter 3 focuses on monitoring types, and Chapter 4 describes general features of analytical methods used in marine monitoring. The book concludes with a discussion about the application of meteorology principles in marine monitoring.

With this book, I hope to continue to “pass the knowledge” that has been gathered by many individuals in the last two decades, with a special tribute to some of my “masters” and friends without whom I would not have developed such an international and multidisciplinary career, in particular Francis Grousset, Olivier Donard and Ben Griepink.

Philippe QUEVAUVILLER

November 2015

Glossary

AAS	atomic absorption spectrometry
ACSV	adsorptive cathodic stripping voltammetry
AE	anion exchange
AED	atomic emission detector
AES	atomic emission spectrometry
AFS	atomic fluorescence spectrometry
APDC	ammonium pyrrolidine dithiocarbamate
ASE	accelerated solvent extraction
ASV	anodic stripping voltammetry
BBD	butyl butylation derivatization
BCR	European Community Bureau of Reference
CCSA	constant current stripping analysis
CCT	capillary cryogenic trap
CE	capillary electrophoresis

(C)GC	(capillary) gas chromatography
CRM	certified reference material
CSC	cathodic stripping chronopotentiometry
CSV	cathodic stripping voltammetry
CT	cryogenic trap; cryotrapping
CV	cold vapor
CVG	chemical vapor generation
CVT	cold vapor technique
D	distillation
dASCP	derivative anodic stripping chronopotentiometry
DGT	diffusion gradients in thin-film
DPASV	differential pulse anodic stripping voltammetry
DPC	diphenylcarbazide
DPCSV	differential pulse cathodic stripping voltammetry
ECD	electron capture detector
EI	electron impact ionization
ESI	electrospray ionization
Et	ethyl
ETAAS	electrothermal atomic absorption spectrometry
Eth	ethylation derivatization
EXAFS	extended X-ray absorption fine structure

FAAS	flame atomic absorption spectrometry
FI	flow injection
FIA	flow injection analysis
FPD	flame photometric detector
FPLC	fast protein liquid chromatography
GC	gas chromatography
HG	hydride generation
HPLC	high-performance liquid chromatography
HR	high resolution
HS	headspace
HTFA	trifluoroacetylacetone
IBMK	isobutyl methyl ketone
IC	ion chromatography
ICP-AES	inductively-coupled plasma atomic emission spectrometry
ICP-MS	inductively-coupled plasma mass spectrometry
ICP-OES	inductively-coupled plasma optical emission spectrometry
ID	isotope dilution
IDMS	isotopic dilution mass spectrometry
IE	ion exchange
IT	ion trap

LA	laser
LC	liquid chromatography
LLE	liquid–liquid extraction
LOD	limit of detection
MALDI	matrix-assisted laser desorption and ionization
MCGC	multicapillary gas chromatography
Me	methyl
MIBK	methyl isobutyl ketone
MIP	microwave induced plasma
MS	mass spectrometry
MTLPs	metallothionein-like proteins
MTs	metallothioneins
MW	microwave
NaDDC	sodium diethyldithiocarbamate trihydrate
OCP	organichlorine pesticide
ORS	octopole reaction system
OTC	organotin compound
PFPD	pulsed flame photometric detector
PLE	pressurized solvent extraction
PLM	permeation liquid membrane
Pr	propylation derivatization

Preconc	preconcentration
PSA	potentiometric stripping analysis
PTFE	polytetrafluoroethylene
PTI	purge and trap injection
QF	quartz furnace atomizer
RP	reversed phase
SCP	stripping chronopotentiometry
SE	size exclusion
SEC	size exclusion chromatography
SF	sector field
SIDMS	speciated isotope dilution mass spectrometry
SPE	solid phase extraction
SPM	suspended particulate matter
SPME	solid-phase microextraction
TFA	trifluoroacetylacetate
TMAH	tetramethylammonium hydroxide
TOF	time-of-life
USN	ultrasonic nebulizer
UV	ultraviolet photolysis (treatment)
UV/vis	ultraviolet/visible

VG	vapor generation
WFD	Water Framework Directive
XANES	sX-ray absorption near-edge spectroscopy
XRF	X-ray fluorescence

Abbreviations

Conventions and legislative units

DIN	Deutsche (German) Industrial Norm
EU	European Union
HELCOM	Helsinki convention for the Baltic Sea
MSD	Marinde Strategy Directive
OSPAR	Oslo-Paris convention for the North Sea
SFD	Shell Fish Directive
WFD	Water Framework Directive

Atomic absorption techniques

AAS	Atomic absorption spectrometry
CVAAS	Cold vapor atomic absorption spectrometry
HGAAS	Hydride generation atomic absorption spectrometry

GFAAS	Graphite furnace atomic absorption spectrometry
AFS	Atomic fluorescence spectrometry
STPF	Stabilized temperature platform furnace
HCL	Hollow cathode lamp
EDL	Electronic discharge lamp

Plasma techniques

ICP-AES/OES	Inductively coupled plasma-atomic/optical emission spectrometry (synonyms)
ICP-HRMS	Inductively coupled plasma high resolution mass spectrometry
ICP-MS	Inductively coupled plasma mass spectrometry
ICP-QMS	Inductively coupled plasma quadrupole mass spectrometry

X-ray techniques

ED-SEM	Energy dispersive scanning electron microscopy
EDXRF	Energy dispersive X-ray
XAS	X-ray absorption spectroscopy
XPS	X-ray photon spectroscopy
XRF	X-ray fluorescence
XRD	X-ray diffraction
PXRF	Portable X-ray fluorescence
PIXE	Particle induced X-ray emission

Electrochemical techniques

ASV	Anodic stripping voltametry
CSV	Cathodic stripping voltametry
PSA	Potentiometric stripping analysis

Quality control

IRM	Internal reference material
CRM	Certified reference material

Marine Monitoring: Historical Background, Regulatory Framework and Science–Policy Interactions

1.1. Introduction

Public awareness about marine pollution and regulatory responses is relatively recent [KRA 11]. The fact that chemical products released into the sea could be hazardous only became “shocking” in the 1950s with the methyl-mercury contamination of tuna and swordfish in Minamata Bay (Japan) in 1956, which resulted in neurological syndromes in the population. Some years later, the wrecking of the Torrey Canyon oil tanker along the Cornish coast (March 1967) and the blowout of an oil platform off the Californian coast in 1969 highlighted the fragility of marine ecosystems in the case of oil spills. The number of dramatic pollution events affecting marine quality would require a full volume but what should be remembered here is that these oil spills resulted in “visible” effects, which led to reactions from the public and changes in opinion that helped create a climate in which legislation was deemed necessary and scientific activities in research and monitoring were encouraged [KRA 11]. Research into the input, transport and fate of pollutants in the marine environment developed in the 1970s, which is reflected by the first issue of the *Marine Pollution Bulletin*, published in January 1970, which aimed to “spread news of pollution” rather than publishing research results, with the objective of informing policymakers [ANO 70].

In 1977, the International Council of Scientific Union's (ICSU's) Scientific Committee on Problems of the Environment (SCOPE) defined "monitoring" as "the collection, for a predetermined purpose, of systematic, inter-comparable measurements or observations in a space–time series, of any environmental variables or attributes which provide a synoptic view or a representative sample of the environment (global, regional, national, or local). Such a sample may be used to assess existing and past states, and to predict likely future trends in environmental features" [HOL 77]. This definition of monitoring, still valid today, turns it into a systematic method of collecting data needed for environmental problem-solving, which is linked to environmental policy [KRA 11].

Monitoring is not only focused on determining concentrations of harmful substances in various compartments of the (marine) environment, e.g. water, sediment and biota. It also includes physical parameters such as salinity, turbidity and pH as well as biological effects [KRA 11]. The basic reasoning behind monitoring has evolved over the years: in the mid-1970s, the focus was on avoiding health hazards, and the knowledge about the fate of pollutants not representing a direct threat to human health was considered to be of lesser importance. Nowadays, monitoring constitutes one branch in a more holistic approach related to marine management and international conventions and regulations (see section 1.3).

Historic developments of marine monitoring (with a focus on the North and Baltic Seas) have been described by Kramer [KRA 11] and will not be repeated here. Only key milestones that have led to current regulations will be described. The first landmark in (marine) monitoring is considered to be the United Nations Conference on the Human Environment that was held in Stockholm in June 1972, which resulted in the adoption of a declaration and an action plan. Principle 7 of the declaration stated that "all possible steps shall be taken to prevent pollution of the seas by substances that are liable to create hazards to human health, to harm living resources and marine life, to damage amenities or to interfere with other legitimate uses of the sea", whereas the action plan recommended that "governments actively support, and contribute to international programs to acquire

knowledge for the assessment of pollutant sources, pathways, exposures and risks” [UNE 72], quoted by [KRA 11]. This conference strengthened the developing environmental (marine) monitoring efforts in various national and international programs, and had some effects on harmonization and structuring marine monitoring plans and activities by active international organizations in the field [KRA 11].

Today, it is well known that marine ecosystems are experiencing unprecedented environmental changes, driven by human activities [ROO 11a]. Issues such as pollution not only from land- and sea-based sources but also from fishing, marine debris, the loss and degradation of valuable habitat and invasions by non-native species are recognized worldwide. However, the initial conventions, and hence research and monitoring, were heavily focused on measurements related to the two major problems at that time: eutrophication and contamination. Furthermore, frequent sampling at sea also became a source of hydrological data in a broader sense, as contaminant data are generally supported by metadata such as salinity, temperature, dissolved oxygen and others. As said above, this has been going on since the late 1960s and early 1970s. Not surprisingly, marine chemical monitoring is presently one of the technically most advanced branches of marine environmental monitoring. Gradually, the focus shifted from pollution to a more holistic approach, often referred to as the ecosystem approach. Chemical monitoring is now only one aspect, albeit an important one, of marine environmental monitoring. Yet, new threats such as climate change and ocean acidification will certainly give a new emphasis on chemical measurements [ROO 11a].

This chapter aims at giving an overview of the existing international organizations and conventions that are central to marine monitoring and research.

1.2. International institutions

Initiatives related to environmental (marine) monitoring were started much before the 1972 Stockholm Conference by a number of international institutions with a certain competition among different

monitoring activities [KRA 11], e.g. a pollution research program developed by the Organisation for Economic Co-operation and Development (OECD) in 1965, marine pollution monitoring activities developed by the International Council for the Exploration of the Sea (ICES) from 1966 onward, etc. In 1969, the UN Food and Agriculture Organization (FAO) convened a “Technical conference on marine pollution and its effects on living resources and fishing”. The same year saw the creation of a joint Group of Experts on the Scientific Aspects of Marine Pollution (GESAMP), cosponsored by the International Maritime Organization (IMO), UNFAO, United Nations Educational, Scientific and Cultural Organization (UNESCO) and World Meteorological Organization. The purpose of this group was to advise the various agencies and UN bodies that were concerned with marine pollution. Later, the World Health Organization, International Atomic Energy Agency and United Nations Environmental Programme (UNEP) joined the initiative [WIN 91]. A strategic vision on scientific aspects of marine environmental protection was developed by this expert group [GES 05].

These developments also resulted in the creation of SCOPE by the ICSU in 1969, which published a report on “Global Environmental Monitoring” [SCO 71] as input for the Stockholm Conference (quoted by [KRA 11]). This report stressed the need for a stronger coordination between a marine pollution monitoring system and Integrated Global Ocean Station System, then under development by the Intergovernmental Oceanographic Commission (IOC) for monitoring the physical conditions of the oceans. Studies based on monitoring of water, superficial sediments and biota for selected critical substances were proposed to be undertaken in pilot areas such as the North Sea, the Baltic Sea, the Mediterranean Sea and the Puget Sound (US). This formed the basis for developing aspects of policy by the IOC and coordinating international science by the Scientific Committee on Oceanic Research (SCOR). Ideas were further developed in the action plan for a Global Environmental Monitoring System [MUN 73] and priority pollutants were defined [AND 88].

1.2.1. *International Council for the Exploration of the Sea*

ICES was founded in 1902. This organization is an internationally recognized player in many scientific aspects dealing with the northern Atlantic Ocean [GRI 03, KRA 11]. Originally, ICES focused mainly on fish and fisheries studies, and the issue of monitoring was hardly touched on within the first 70 years of its existence [WEN 72], which changed from 1970s onward [ROZ 02, GRI 03, KRA 11]. Initially, stimulated by an OECD program on pollution research proposed to ICES leaders in 1965, a cooperation between ICES, IOC and OECD was established, leading to the establishment of the ICES Fisheries Improvement Committee in 1966 (from which, in 1978, the Marine Environmental Quality Committee was formed), and 2 years later the ICES Working Group on Pollution of the North Sea, followed in 1971 by the ICES/SCOR joint Working Group (WG) on the Study of the Pollution of the Baltic [KRA 11].

Surveys have been carried out by ICE, which can be considered a kind of precursor to regular monitoring programs [KRA 11], examples of which are baseline studies on trace contaminants in fish and shellfish in the North Sea [ICE 74], the Baltic Sea [ICE 77a] and the northern Atlantic Ocean [ICE 77b]. Following these baseline studies, an annual North Sea Monitoring Programme was initiated in 1974 [ICE 77c], followed by the Baltic Monitoring Programme (BMP) in 1979 [KRA 11]. These studies and programs were associated with the establishment (in 1973) of the ICES Advisory Committee on Marine Pollution, which developed guidelines on sampling, sample preparation, analytical procedures and data reporting required to obtain good quality data for various objectives, such as public health and environment protection or trend monitoring. This committee produced annual reports until 1992, and then was transformed into the Advisory Committee on the Marine Environment, which produced annual reports until 2003 [KRA 11].

Besides monitoring activities, ICES was active in method developments and intercalibration exercises through various WGs, several of which had an influence on the design and operation of monitoring studies (e.g. WGs on marine chemistry, marine

ssediments and biological effects of contaminants). From 1984 to 1998, the data from the (Oslo and Paris, OSPAR) Joint Monitoring Programme (JMP) (see below) have been compiled and quality checked by ICES, followed by Helsinki Commission (HELCOM) data [KRA 11]. This organization has thus been, and still is, instrumental for the development of marine monitoring programs, first initiating baseline surveys and then supporting monitoring programs carried out by national organizations under the OSPAR and HELCOM umbrella.

1.2.2. United Nations Environment Programme

UNEP was established by the UN General Assembly as a follow-up to the 1972 Stockholm Conference on the Human Environment (see <http://www.unep.org>) to serve as a focal point for environmental action and coordination within the UN system [KRA 11, ROO 11a]. One of the first priority areas was the assessment and control of marine pollution [BIR 74], with a focus on coastal areas and semi-enclosed seas through the Regional Seas Programme initiated in 1974; it provides a legal, administrative, substantive and financial framework for the implementation of Agenda 21 [UN 92], the Plan of Implementation of the World Summit on Sustainable Development [UN 02] and for the Bali Strategic Plan [UNE 04]. It is an action-oriented program and focuses not only on the mitigation or elimination of the consequences but also on the causes of environmental degradation (<http://www.unep.ch/regionalseas>). The action plans are usually adopted by high-level intergovernmental meetings and implemented, in most cases, within the framework of a legally-binding Regional Seas Convention and its specific protocols, under the authority of the respective contracting parties (e.g. MEDPOL, see section 1.3.6). The focus has gradually shifted from protecting the marine environment from pollution to striving towards sustainable development of the coastal and marine environment through integrated management. Currently, 17 members of the regional seas family are reflected in the Assessment of Assessment (AoA) regions (see below). Altogether more than 140 countries participate in at least one Regional Seas Action Plan or convention. In 12 of the regions, states have also adopted a legally-binding convention [ROO 11a]. Most of the regions covered by this program are located in

less developed countries, but some partner programs, e.g. the Baltic (HELCOM) and the North-East Atlantic (OSPAR), are also members. The first plan was adopted in 1975, involving 16 Mediterranean countries and the European Community to form the Mediterranean Action Plan (MAP), which is still in operation (www.unepmap.org). The approach and strategy followed include an environmental assessment and the implementation of regional seas monitoring programs [GER 94]. Prospects for global ocean pollution monitoring were discussed in the early 1980s and were not followed up by concrete developments, owing to the low levels of contaminants, the near impossibility of identifying biological effects, logistical problems related to true open ocean monitoring and associated costs [UNE 84]. Marine regions are those identified by the UNEP Regional Seas and in the AoA [UNE 09]. An overview of the major marine regions is graphically illustrated in Figure 1.1. For most of these regions, agreements were made between the parties involved and environmental monitoring was initiated in support of these policies [ROO 11a]. An overview of prominent regional organizations is presented in Table 1.1.

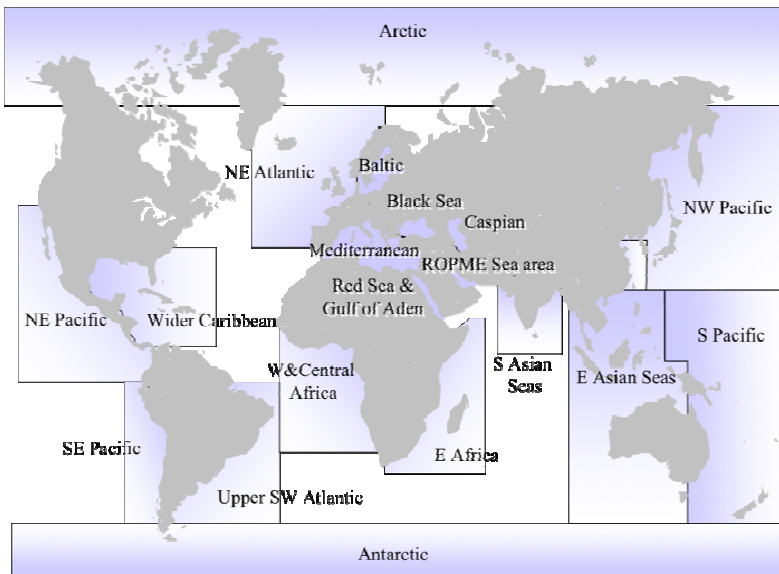


Figure 1.1. UNEP regional seas (from [ROO 10])

Regional commission or organization	Website
Permanent Commission for the South Pacific (CPPS)	http://www.cpps-int.org
South Asia Co-operative Environment Programme (SACEP)	http://www.sacep.org
South Pacific Regional Environment Programme (SPREP)	http://www.sprep.org
Convention on the Conservation of Antarctic Marine Living Resources (CCAMLR)	http://www.ccamlr.org
Caribbean Environment Programme (CEP)	http://www.cep.unep.org
Protection of the Arctic Marine Environment (PAME)	http://www.pame.is
Baltic Marine Environment Protection Commission (Helsinki Commission or HELCOM)	http://www.helcom.fi
Commission for the Protection of the Black Sea Against Pollution	http://www.blacksea-commission.org
Caspian Environment Programme (CEP)	http://www.caspianenvironment.org
East African Coastal Database	http://www.unep.org/eafatlas
Mediterranean Action Plan (MAP)	http://www.unepmap.org
OSPAR Commission (OSPAR)	http://www.ospar.org
The Regional Organization for the Conservation of the Environment of the Red Sea and Gulf of Aden (PERSGA)	http://www.persga.org
Regional Organization for the Protection of the Marine Environment (ROPME)	http://www.ropme.net

Table 1.1. Overview of the major regional commissions or organizations and their websites (adapted from [ROO 11a])

The new Regional Seas strategy encourages the Regional Seas programs to increase monitoring and assessment activities, and to facilitate a science-based decision-making system including participation in such processes of the UN General Assembly known as the Global Assessment of the State of the Marine Environment. This concept, i.e. establishing regular marine environment assessments, was initiated in 1999 at the seventh session of the Commission on

Sustainable Development. It has led to the AoA which is described in detail in section 1.3 [ROO 11a]. UNEP is one of the lead agencies responsible for taking forward the AoA through the implementation of UN General Assembly Resolution 60/30. UNEP is also responsible for the secretariat set up to implement the 1995 Global Programme of Action for the Protection of the Marine Environment from Land-Based Activities and, one of the implementing agencies, for the Global Environment Facility (GEF). This is an independent international financing entity with the long-term goal to ensure progress toward global environmental security. The UNEP portfolio of GEF-funded activities in international waters includes global assessments, transboundary diagnostic analyses of shared water bodies, support for the implementation of strategic action programs for marine and freshwater areas, and support for integrated management of shared freshwater bodies. Because water issues play an important and increasing role in international development cooperation, GEF has designated international waters as one of its four focal areas [ROO 11a]. The Global International Waters Assessment (GIWA), led by UNEP and 50% funded by GEF, will provide the information needed for GEF's work in this particular area. The aim of GIWA is to produce a comprehensive and integrated global assessment of international waters, the ecological status of and the causes of environmental problems in 66 water areas around the world, and to focus on the key issues and problems faced by the aquatic environment in transboundary waters. The assessment is designed not merely to analyze the current problems but also to develop scenarios of the future conditions of the world's water resources and analyze policy options with a view to providing sound scientific advice to decision makers and managers concerned with water resources. In the near future, GIWA activities will be linked and coordinated with the monitoring programs described elsewhere in this section, such as OSPAR and HELCOM.

UNEP should essentially be seen as a facilitator and does not play the same role as organizations such as OSPAR and HELCOM (see sections 1.3.3 and 1.3.4). UNEP's role is to create the conditions that make marine monitoring feasible through capacity-building projects, technical and scientific advice, e.g. in the form of technical

guidelines, and by bringing organizations together that have common goals, facilitating exchange of practices and information [ROO 11a].

1.2.3. *Intergovernmental Oceanographic Commission of UNESCO*

The Intergovernmental Oceanographic Commission (IOC) was established by UNESCO in 1960. It promotes and coordinates international cooperation and programs in marine research, services, observation systems, hazard mitigation and capacity development in order to learn more and better manage the nature and resources of the ocean and coastal areas. In addition, the Commission strives to further develop ocean governance, which necessitates strengthening the institutional capacity of member states in marine scientific research and of ocean management (www.ioc-unesco.org). In short, it is the designated UN entity for coordinating global ocean sciences [ROO 11a]. As a follow-up of the 1972 Stockholm Conference, the IOC developed the “Programme of Global Investigation of Pollution in the Marine Environment” (GIPME), cosponsored by UNEP and the IMO, the objectives of which were to provide authoritative evaluations of the state of the marine environment at both regional and global levels to identify the requirements for measures to prevent, or correct, marine pollution, and to develop/implement procedures for assessing and improving compliance and surveillance monitoring of conditions and effects in the marine environment [KRA 11]. The GIPME is based on a regionally-based marine pollution monitoring system known as MARPOLMON [KUL 1986], which constitutes a marine chemical component of Global Environmental Monitoring System [AND 88]. Important GIPME priorities are the baseline studies (status) and the standardization of methods and techniques. A great deal of emphasis has been placed on developing, testing and calibrating methodologies to ensure the quality of data for the major classes of contaminants measured in a variety of marine phases and to attempt to determine fluxes in the marine environment [DAW 88]. The day-to-day work within the GIPME is conducted by three expert scientific groups: the Group of Experts on Methods, Standards and Intercalibration in charge of the assessment and methodology for measuring levels and flux of contaminants; the Group of Experts on the Effects of

Pollutants to study the biological effects of contaminants, pollution assessment and indicators of biological and ecosystem condition on the marine environment; and the Group of Experts on Standards and Reference Materials, which deals with the assurance of data quality and comparability of measurements.

Furthermore, the IOC manages the Global Ocean Observing System, the ocean component of the Global Climate Observing System. As such, it helps to improve operational oceanography, weather and climate forecasts and monitoring and supports the sustained observing needs of the UN Framework Convention on Climate Change. The vision guiding the development of a Global Ocean Observing System is one of a world served by a unified global network providing the information needed by governments, industry, science and the public to deal with marine-related issues, including environmental issues and the influence of the ocean upon climate [ROO 11a]. The International Oceanographic Data and Information Exchange program enhances the IOC marine research and management programs by facilitating the exploitation, development and exchange of oceanographic data and information between participating member states and by meeting the needs of users for data and information products [ROO 11a].

1.2.4. European Union

The first formal environment policy was adopted by the then named European Economic Community in 1972. As such, the European Union (EU) does not carry out monitoring activities but sets the legal framework of environment monitoring through regulations (Directives and Decisions), which have to be implemented by member states. Various legal instruments to limit the pollution of the aquatic environment have been put in place since the early 1970s, a number of which have been combined (some of them repealed) by the EU Water Framework Directive (WFD) [EUR 00] adopted in December 2000. Although the main purpose of this directive is to establish a framework for the protection of inland surface and ground waters, the WFD also covers surface “transitional waters” (such as estuaries) and coastal waters up to one nautical mile from the coastline

[FER 07]. Among other milestones (e.g. risk assessment, impact studies, programs of measures), the Directive includes obligations for member states to establish and implement monitoring of “water status” (for surface waters, ecological and chemical status) based on different criteria and parameters [QUE 11b]. The “chemical status” is linked to compliance to Environmental Quality Standards (EQS) established for 33 priority substances under the “daughter” Directive to the WFD, namely the Directive on Priority Substances [EUR 08a], which has recently been amended to include new substances. The main legal instrument concerning the marine environment is the Marine Strategy Directive, which has been described in depth by Verreet [VER 11] and is summarized in section 1.4.

1.3. International conventions/programs

Combating marine pollution requires international cooperation efforts that have been orchestrated by a number of international treaties ratified over the past 30 years. Kramer [KRA 11] and Roose [ROO 11a] give a snapshot of a number of international conventions that have become instrumental in marine protection based on monitoring programs and trend studies of the marine environment. The present section gives a summary of these conventions.

1.3.1. *UN Convention on the Law of the Sea*

At the global level, the UN Convention on the Law of the Sea (UNCLOS) provides a legal framework and basic principles for the management of the oceans [UNE 09]. Ocean issues are considered in a comprehensive manner in the United Nations General Assembly and its processes. The international rules and standards that implement UNCLOS provisions are further developed by specialized global organizations such as FAO and IMO. The instruments can be both conventions (e.g. MARPOL 73/78, see section 1.3.5) and normative instruments (e.g. Code of Conduct for Responsible Fisheries). A large number of multilateral environmental agreements also apply to the oceans, covering themes such as climate change, hazardous substances, biodiversity and protection of species and habitats.

UNCLOS also provides the framework for regional seas collaboration. The regional level is appropriate for responding to the many problems that occur at larger than national scales. Regional organizations can bring together coastal states adjacent to the same oceans and seas, and sometimes also other states that use the areas. The most important regional seas conventions and organizations are discussed below but we should realize that in some oceans and seas there are no strong instruments or collaboration. Alternatively, in areas such as North America, bilateral cooperation can be appropriate [ROO 11a].

1.3.2. London Dumping Convention

The “Convention on the Prevention of Marine Pollution by Dumping of Wastes and Other Matter”, in short the “London Dumping Convention” or “London Convention” (LC 72), was adopted in 1972 and enforced in 1975. This international treaty established an approach based on a “black list” (chemicals to be banned) and a “gray list” (chemicals for which dumping is to be restricted) to regulate ocean dumping. A permanent secretariat is hosted by the IMO.

1.3.3. OSPAR conventions

The same year (1972), the “Convention for the Prevention of Marine Pollution by Dumping from Ships and Aircraft”, known as the “Oslo Convention”, was signed and enforced 2 years later. Its framework covered the North-East Atlantic and part of the Arctic Ocean, but excluded the Baltic Seas [KRA 11]. Similarly to LC, the Oslo Convention made a distinction between “black” and “gray” list chemicals; it was complemented by the “Convention for the Prevention of Marine Pollution from Land-Based Sources”, known as the “Paris Convention”, which was adopted in 1974 and enforced in 1978. The Oslo Commission (OSCOM) and the Paris Commission (PARCOM) shared a joint secretariat in London (OSPARCOM) [OSP 84]. This framework evolved into the “Convention for the Protection of the Marine Environment of the North-East Atlantic” or “OSPAR Convention” that was signed in 1992 (enforced in 1998), which is the current legislative instrument regulating international

cooperation on environmental protection in the North-East Atlantic [KRA 11]. It combines and updates both the above-mentioned OSPAR Conventions. Activities carried out under the Convention are managed by the OSPAR Commission. The OSPAR Convention now regulates – for its geographic region – European standards on marine biodiversity, eutrophication, the release of hazardous and radioactive substances into the seas, the offshore oil and gas industry and baseline monitoring of environmental conditions [TRO 94].

In order to examine the conditions of the sea covered by the conventions, OSCOM and PARCOM established a permanent Joint Monitoring Group (JMG), and with the guidance of ICES, a JMP in 1978 [OSP 84]. OSPARCOM adopted many of the principles of the ICES program in defining the JMP [KRA 11]. The JMG monitoring program had the following four main objectives [POR 86], i.e. the assessment of,

- 1) possible hazards to human health;
- 2) harm to living resources and marine life;
- 3) the existing levels of marine pollution (spatial distribution);
- 4) the effectiveness of measures taken for the reduction of marine pollution within the framework of the conventions (temporal trend assessment).

The JMP was based on the national programs of the contracting parties, with their national laboratories responsible for the sampling and analyses. To ensure comparability of data, calibration of methods had to be supported by participation in (e.g. ICES) interlaboratory comparison studies. The actual monitoring program started in 1979, and was initially limited to mercury and cadmium in seawater and in organisms, and polychlorinated biphenyls (PCBs) in organisms. Sampling frequencies were set according to monitoring objectives (see [KRA 11]).

In 2003, the Ministerial Meeting of the OSPAR Commission adopted a strategy for the Joint Assessment and Monitoring

Programme (JAMP), a combination of the national monitoring programs of the contracting parties. This provided a framework for work to prepare and produce a series of thematic assessments (quality status report, QSR). Thus, OSPAR is coordinating repeated measurement and assessment of the marine environment over a decadal time frame [KRA 11]. The organizational structure of OSPAR changed in 1995. Monitoring and assessment became a task of the Assessment and Monitoring Committee of OSPAR (ASMO). Monitoring has since been split into several domains in several working groups under ASMO, such as the Working Group on Concentrations, Trends and Effects of Substances in the Marine Environment (SIME), WG INPUT, dealing with atmospheric inputs (via the Comprehensive Atmospheric Monitoring Programme) and riverine inputs and discharges (via the Comprehensive Study of Riverine Inputs and Direct Discharges), and WG MON, e.g. via the Co-ordinated Environmental Monitoring Programme (CEMP) [DE 06].

The QSR 2000 [OSP 00] was based on the combined efforts of JMP and JAMP. The geographic coverage was expanded to a larger area, the North-East Atlantic (the OSPAR convention area) which was subdivided into five regions (Arctic waters, Greater North Sea, Celtic Seas, Bay of Biscay and Iberian Coast and Wider Atlantic). For each region, a separate report was prepared as well as a holistic synthesis report for the entire area. The most recent QSR 2010 was launched at the occasion of the Ministerial Meeting of the OSPAR Commission in Bergen (2010). Again a regional approach was followed in a printed and electronically available version (<http://qsr2010.ospar.org>), albeit in one volume [OSP 10].

OSPAR has taken, with its assessment tools, the next step in data assessment by using novel statistical approaches to test against set reference values such as background assessment concentrations and environmental assessment criteria. OSPAR also has well-defined statistical tools for temporal trend analysis that takes the quality of the data used into account. This has resulted in innovative and high-quality assessments that appear at regular intervals describing, e.g.,

the spatial distribution and trends of contaminants in the OSPAR area. Like HELCOM, OSPAR has developed ecological quality objectives (EcoQOs) for the implementation of the ecosystem approach but there are also no legal sanctions if contracting parties do not meet their obligations, resulting in gaps in the datasets [ROO 11a].

1.3.4. Helsinki Convention

International cooperation in the study of the Baltic Sea environment can be traced back to the establishment of ICES in 1902, which is mentioned earlier [ROO 11a]. This led to the development of a parallel framework to the OSPAR Convention in the Baltic Sea, resulting in the adoption of the Convention on the Protection of the Baltic Sea Area in 1974, known as the “Helsinki Convention” (enforced in 1980), which is governed by the HELCOM (or Baltic Marine Environment Protection Commission). The main goal of HELCOM is to protect the marine environment of the Baltic Sea from all sources of pollution, and to restore and safeguard its ecological balance. The present contracting parties to HELCOM are Germany, Denmark, Estonia, Finland, Latvia, Lithuania, Poland, Russia and Sweden. The setup is very similar to that of OSPAR (see below), and many of the principles – such as the “best environmental practices”, “best available technologies” and “the polluter pays” – are adopted and applied by HELCOM [ROO 11a]. Following a review of its first 20 years of existence [HEL 94], this convention was updated in 1992 by a new framework enforced in 2000, which now covers the whole of the Baltic Sea area, including inland waters as well as the water of the sea itself and the seabed. Measures were also taken in the whole catchment area of the Baltic Sea to reduce land-based pollution. All these conventions have aimed at the regulation of inputs to carry out baseline studies (present status), monitor for trends and carry out intercalibrations between contracting parties to warrant quality data [KRA 11].

Under the Helsinki Convention, monitoring of physical, chemical and biological variables of the open sea started in 1979 (radioactive

substances in 1984) but was considered a national obligation. It was called BMP and revised several times. The first pilot period covered 1979–1983; the second phase (1984–1988) had a larger coverage. The third stage started in 1989. For political reasons, the coastal areas of the sea were only poorly covered by the BMP, and the program focused on the open sea. The aim of the BMP was to monitor the long-term changes in selected indicators in the Baltic ecosystem, details of which are given by Kramer [KRA 11]. The Cooperative Monitoring in the Baltic Marine Environment (COMBINE) was instituted in 1992 with the aim of quantifying the state, impacts and changes in the various compartments (water, biota and sediment). The “ecosystem approach” adopted by the Joint HELCOM/OSPAR Ministerial Meeting in 2003 (including EcoQOs and related indicators) led to a different type of assessment focused on the pressures of human activities as well as the resulting impacts on, and state of, the marine environment.

1.3.5. MARPOL

Recognizing the threat of pollution of the seas by oil from shipping, the International Convention for the Prevention of Pollution of the Sea by Oil, or the “OILPOL Convention”, was adopted in 1954 (enforced in 1958), primarily addressing pollution resulting from routine tanker operations. Later, the Intergovernmental Maritime Consultative Organisation (IMCO, since 1982 called IMO) organized the International Conference on Marine Pollution in London in 1973, which led to the International Convention for the Prevention of Pollution from Ships, known as MARPOL [KRA 11]. This convention was revised by the MARPOL protocol, the combination of which led to the MARPOL 73/78 Treaty adopted in 1978 and enforced in 1983. Its worldwide objective was to preserve the marine environment through the complete elimination of pollution by oil and other harmful substances and the minimization of accidental discharge of such substances. The initial focus on oil was expanded in later years with the inclusion of other substances: noxious liquid substances

carried in bulk; harmful substances carried in packaged form; sewage, garbage and air pollution [KRA 11].

1.3.6. Mediterranean Sea: Barcelona Convention

In 1976, the Mediterranean countries and the EU adopted the Barcelona Convention for the Protection of the Mediterranean Sea Against Pollution (see [ROO 11a]), overarching the MAP, approved the year before (see <http://www.unepmap.org/>). This framework convention includes the preparation of technical protocols, such as the protocols for the protection against pollution from land-based sources [BAR 80] and from hazardous waste disposal [BAR 96]. As the environmental assessment component of MAP and the associated protocols, the Programme for the Assessment and Control of Pollution in the Mediterranean region (MEDPOL) was established. When started in 1975, its main aim was the establishment of a network of institutions undertaking marine pollution work and the collection of information regarding the level of pollution in the Mediterranean Sea. The monitoring activities covered heavy metals in marine biota (mainly mercury and cadmium), halogenated hydrocarbons in marine biota (mainly PCBs and dichlorodiphenyltrichlorethane (DDTs)) and petroleum hydrocarbons in seawater. The development and maintenance of these national monitoring programs were the aim of the second phase (1981), whereas more recently (1996) the emphasis has shifted from pollution assessment to pollution control. In parallel, MEDPOL provides assistance in the formulation and implementation of regional and national action plans addressing pollution from land-based sources and activities. It also formulates and carries out capacity-building programs related to the analysis of contaminants and treatment of data and to technical and management training [ROO 11a]. Although monitoring of the Mediterranean environment is firmly in place for several countries, a coordinated and well-developed monitoring program has not been realized, as is the case for HELCOM and OSPAR. Emphasis has been mainly on capacity-building and setting up the conditions that will eventually result in a sustainable monitoring program for the Mediterranean. For instance, guidelines have been developed covering various aspects of

monitoring and efforts have been made to enhance the quality of the processes [ROO 11a].

1.3.7. Bonn Agreement

Another international framework (cited by [KRA 11]) on discharges of oil and other substances into the North Sea region under the Agreement for Cooperation in Dealing with Pollution of the North Sea by Oil, or the “Bonn Agreement”, was adopted and enforced in 1969 and later superseded by the Agreement for Cooperation in Dealing with Pollution of the North Sea by Oil and other harmful substances (1983, Bonn Agreement; in force 1989). Now parties were required to jointly develop and establish guidelines for joint action and to provide information on pollution incidents. Developments were discussed on the occasion of its 40th anniversary (Bonn Agreement, 2009). One of the implementation instruments of the Bonn Agreement is the on-going aerial surveillance program, which started in 1986, to monitor and assess trends in levels of oil input into the marine environment [CAR 07].

1.3.8. Arctic Ocean: Arctic Monitoring and Assessment Programme

For the Arctic Ocean, data are gathered and assessed through the Arctic Monitoring and Assessment Programme (AMAP) [ROO 11a]. AMAP was established in 1991 to implement certain parts of the Arctic Environmental Protection Strategy, primarily “providing reliable and sufficient information on the status of, and threats to, the Arctic environment, and providing scientific advice on actions to be taken in order to support Arctic governments in their efforts to take remedial and preventive actions relating to contaminants” (see <http://www.amap.no>). The Arctic Council, established in 1996 by the eight Arctic countries (Canada, Denmark, Finland, Iceland, Norway, Russia, Sweden and the United States), coordinates AMAP activities. AMAP was conceived as a program that integrates both monitoring and assessment activities in relation to pollution issues and provides information and reports on the state of the Arctic environment. The

AMAP Trends and Effects Monitoring Programme [AMA 00] is designed to monitor the levels of pollutants and their effects in all compartments of the Arctic environment [ROO 11a]. The program includes both monitoring and research components, and special studies that yield information that is vital for the valid interpretation of monitoring data. AMAP has produced comprehensive and scientifically sound assessments of contaminants in the Arctic environment, discussing their levels, trends, and effects (both based on comparison with current literature and the observation of biological effects as such) [AMA 02]. An AMAP assessment should therefore not be considered as a formal environmental risk assessment [ROO 11a]. Rather, it constitutes a compilation of current knowledge. The emphasis is very much on science as the basis for relevant policy and the program has no regulatory purpose as such. The setup is therefore much like a scientific program and there seem to be no legally-binding obligations or a strict set of guidelines as, for instance, there are for the OSPAR CEMP (see further). Specific for this program is the assessment of human health through the study of dietary intake and body burdens [AMA 09].

1.3.9. North East Pacific Ocean

There are extensive chemical data series that cover the North East Pacific region's biophysical environment during much of the past 50 years, particularly off the coasts of the United States and Canada [ROS 09]. These data are, amongst others, the result of directed research and monitoring efforts of governments and academia that cover both ocean physics and chemistry. There is also a well-developed science program for the region. However, there is no formal convention and accompanying monitoring program covering the entire region, as is the case for, the North East Atlantic for example [ROO 11a]. For the northern part of the North East Pacific region, periodic monitoring and assessments are conducted by the Fisheries and Oceans and Environment Canada (DFO), the US National Oceanic and Atmospheric Administration (NOAA), the US Environmental Protection Agency (US-EPA) and their counterparts. A good example is the NOAA's National Status and Trends monitoring program that has been going on since 1986 and covers the Atlantic,

Pacific and Gulf coasts of the United States. Canada, Mexico and the United States also have formal scientific advisory bodies that conduct assessments and provide advice to the respective governments on policy and management [ROS 09]. For the remainder of the region, a UNEP Regional Seas Programme is based on the Convention for Cooperation in the Protection and Sustainable Development of the Marine and Coastal Environment of the Northeast Pacific (Antigua/Guatemala Convention), and was signed in 2002 [ROS 09].

1.3.10. North West Atlantic Ocean

As the North West Atlantic Ocean region is only bordered by the shores of three countries (Canada, Denmark (Greenland) and the United States), no regional convention and accompanying monitoring and assessment program have been set up [ROO 11a]. Nevertheless, there are extensive data series covering most of the past 50 years on the biophysical environment and there is a well-developed science program for the region, including cooperative Canada–US studies on various topics [RIC 09]. Very extensive data collection systems are operating in both countries, including research surveys operating year-round and newly-developed ocean observing systems, which are coming on stream. As for the previous region, a substantial body of assessment work is being carried by the responsible national institutions (DFO, NOAA, US-EPA) or in collaboration with regional organizations. This is particularly the case for the northern part of the region that is also covered by AMAP. There are also strong links with European efforts through ICES (see section 1.2.1). Still, despite its size, the region is mostly approached on a national level. It is therefore not surprising that there has been no overall synthesis of the information on the North West Atlantic Ocean region ecosystems [ROO 11a]. This does not mean that there are no major assessments for the ecosystems along the coast under the auspices of government agencies. For instance, there are Canadian series on the state of the oceans or the United States Coastal Condition Report. Synthesis studies such as the latter give a broader overview of the entire coast but at much lower resolution. Integrated ecosystem assessments are in progress in both countries.

1.3.11. North Sea conferences

The baseline studies, other surveys and surveillance programs, and serious concern at the political level, led to a series of North Sea conferences from 1984 onward [SKJ 06], which were supported by QSR providing an integrated assessment of the cumulative and relative impact of all human pressures on the marine environment, identifying where action needed to be taken [KRA 11]. One of the outcomes of the QSR 1987 was the recognition that despite the large number of contaminants measured, the spatial coverage of the North Sea was rather limited. As a result, the North Sea Task Force (NSTF) was set up, cosponsored by OSPARCOM and ICES, the objectives of which included advice on research and on the implementation of a quality-assured monitoring program [HOO 91]. The aims of the NSTF Monitoring Master Plan were to enhance scientific knowledge and understanding of the North Sea environment, and to overcome shortcomings in data on the distribution of contaminants [REI 90]. The NSTF was active from 1988 to 1994 and its approach was then incorporated into ASMO. Worth noting is that it contributed to the launching of the QUASIMEME proficiency testing scheme [WELL 97], which is described in Chapter 2.

1.3.12. Other conventions

Besides the Barcelona Convention (see section 1.3.6), the concept of the OSPAR Conventions was used as a basis for developing a framework for the protection of other (European) sea areas, such as the Convention on the Protection of the Black Sea against Pollution, the “Bucharest Convention” (adopted in 1992; enforced in 1994).

1.4. The EU marine strategy

Protection of water is a long-standing part of the European Community’s environmental policy¹ [VER 11]. Like the international

¹ With the entry into force of the Lisbon Treaty, the EU is the successor of the European Community. An overview of the current body of EU legislation in the field of water protection is given at http://europa.eu/legislation_summaries/environment/water_protection_management/index_en.htm.

legal framework generally, the European Community's policy regarding the protection of the marine environment has grown in a relatively piecemeal fashion over the years. Quality standards for different types of water use (e.g. shellfish water and bathing water) or seafood were set in a first wave of EC directives on water quality. It was also attempted at the EC level to elaborate common discharge standards for point sources of listed substances on land (Directive 76/464/EC). Over the period 1975–1993, the EC had become, as a regional economic integration organization, a party to the regional sea conventions in three of the four main sea basins around Europe², with discussions regarding membership of the convention dealing with the pollution of the Black Sea (Bucharest Convention) progressing only slowly since the EU membership of Romania and Bulgaria in 2007 [VER 11]. Environmental policy is a shared competence between the EU and its Member States. In contrast, the Conservation of Marine Biological Resources under the Common Fisheries Policy (Treaty on the Functioning of the European Union, Art. 3 (1)(d)) has been an exclusive community policy since the early 1980s, following international developments on access to fisheries resources during the 1970s when coastal states started to declare their exclusive economic zones under UNCLOS (see section 1.3.1). EU Member States agreed that the management of the fisheries stocks in their exclusive economic zone could be better managed in a common European regime.

An important milestone and precedent for a comprehensive approach to the protection of the marine environment was the adoption of the Water Framework Directive 2000/60/EC (WFD) [EUR 00] whose objective is to establish a framework for the protection of inland surface waters, transitional waters, coastal waters and groundwater. This regulation contributes to the achievement of the objectives of international agreements described in section 1.3. Its geographical limit of application (as defined by the

² In the Baltic: the EC became a party to the 1992 Helsinki Convention in 1993. In the North-East Atlantic Ocean: the EC became a party to the 1974 Paris Convention in 1975; this Convention preceded the 1992 OSPAR Convention currently in force. In the Mediterranean Sea, the EC became a party to the 1976 Barcelona Convention in 1977.

term “surface waters”) at the seaward side extends fully to the coastal waters (with a limit of 1 nautical mile from the baseline) and to the territorial waters only with regard to “chemical status” (most often these cover waters up to 12 nautical miles from the coast).

Even after the adoption of the WFD, EU environmental policy makers considered there was a lack of strategy underpinning the policies to protect the marine environment. A commitment to formulate such a strategy was included by the European Parliament and the Council in the sixth Environmental Action Programme adopted in July 2002 and applicable for the period 2002–2012 [EUR 02a]. The European Marine Strategy developed over the period 2002–2005 [EUR 06a] and resulted in the adoption of the Marine Strategy Framework Directive (Directive 2008/56/EC). Marine environmental and maritime policy are, however, not the only relevant policy frameworks for chemical monitoring of the seas. Prevention of pollution by chemicals is also addressed by extensive legislation on production, marketing and use of substances, such as the REACH Regulation [EUR 06b]. An analysis within the framework of the OSPAR Commission concluded in 2008 that most of the EU chemicals legislation seemed to adequately address the possible risk to the marine environment, although this judgment was not fully reached for substances used as veterinary medicines and pesticides [OSP 08].

1.4.1. The notion of “good environmental status”

The objectives of the Marine Strategy Framework Directive (MSFD) are clearly to achieve a certain desired degree of environmental quality through the achievement of a “good environmental status”, which marks an important shift toward a more proactive and forward-looking marine environmental protection regime [VER 11]. It embodies an important aspect of the ecosystem-based approach. The environmental management will be geared to achieving explicit objectives rather than being driven only by the principles and concepts of the past, such as “prevention of pollution”. It can be argued that these past concepts were in themselves

insufficient to secure the maintenance of healthy, clean and productive seas. This shift is not without its own challenges: it hinges on the ability to ensure that the objectives are well formulated and that they provide the correct drivers for management action. Being able to measure and monitor the right variables is a key consideration underlying environmental objective formulation. Details about definitions and qualitative descriptors of “good environmental status” are provided by Verreut [VER 11] and will not be repeated here. Implementation of good environmental status objectives closely relies on the development of operational “criteria and methodological standards”, which is subject to a Commission decision [EUR 10]. This framework provides further guidance on adequate methods by which Member States can ensure that the normative aspects of “good” are correctly applied in their concrete, operational, expression of what “good environmental status” means for their marine waters. The incorporation, under EU law, of these components of the ecosystem-based approach creates a significant driver for the further development and application of quality assessment techniques, which were hitherto only applied in a “soft law” context, such as the EcoQOs promoted earlier in the frameworks of the North Sea Conferences, OSPAR and their equivalents under HELCOM (see section 1.3). The “determination” of good environmental status by the Member States will be accompanied by the setting of targets and associated indicators, which will have to be designed so as to guide the selection of necessary management measures. It is good practice to formulate policy objectives and indicators together, so that there is a clear understanding of how success or failure will be evaluated [VER 11].

1.4.2. Marine strategies of the Member States

The initial assessment, the determination of “good environmental status” and the setting of associated targets and indicators are an extensive prelude to the real substance of the Member States’ marine strategies, which should be their program of measures that are necessary to achieve the environmental objectives. The different steps

have been described by Verreert [VER 11] and are summarized in Table 1.2.

Marine strategy element	First version ready
– A description and assessment of current environmental status, including the environmental impact of human activities	By 15 July 2012
– The determination of good environmental status	By 15 July 2012
– The establishment of environmental targets and associated indicators	By 15 July 2012
– The establishment and implementation of a monitoring programme for ongoing assessment and regular updating of targets	By 15 July 2014, except where otherwise specified in the relevant community legislation
– The programme of measures toward good environmental status	Established by 2015; entry into operation by 2016

Table 1.2. *Documents that together form a “marine strategy” (adapted from [VER 11])*

Similarly to the EU WFD [EUR 00], the Marine Strategy Directive follows an “adaptive management” approach, which takes account of the progress in available knowledge and changing challenges and circumstances, through a 6-yearly review cycle of each of the marine strategy elements [VER 11]. In view of the synergies between Member States’ activities under the regional sea conventions and the MSFD implementation, joint actions are now currently undertaken and programed at EU subregional and national levels in close cooperation (regarding exchanges of data, information and best practices) among the different organizations.

1.4.3. Monitoring in the MSFD policy context

During the development of the EU Marine Strategy, many stakeholders and the European Commission emphasized the need for an “evidence-based policy” and a “knowledge-based approach” [EUR 05], requiring *inter alia* a strong basis in science for all elements of marine strategies. An adequate science–policy interface is

needed so that science continuously informs policy (this is discussed in section 1.5). Only then can the information value derived from observation and monitoring be maximized.

Within the MSFD, monitoring is related to the “on-going assessment of the environmental status of [...] marine waters”, which is based to an important degree on indicators updated on a regular basis [VER 11]. In this context, an environmental indicator is defined by the OECD as “a parameter, or a value derived from parameters, that points to, provides information about and/or describes the state of the environment, and has a significance extending beyond that directly associated with any given parametric value. The term may encompass indicators of environmental pressures, conditions and responses” (OECD online glossary of statistical terms). Indicators are closely linked to the DPSIR (drivers, pressures, state, impact, response) model, i.e. used for risk assessment, pressure analyses, status assessment and responses. A matrix showing the combination of MSFD requirements and the functional relation to elements of the DPSIR framework is given in Table 1.3. “State” is the central component on which MSFD effectiveness should ultimately be assessed [VER 11].

MSFD Article	Additional requirement	Driving forces	Pressures	State	Impact	Response
Art. 8: (Initial) assessment	on basis mainly of Annex III	Descriptive	Descriptive	Descriptive (*)	Descriptive	Descriptive
Art. 9: Good Environmental Status	on basis of EU common criteria and methodological standards	(unlikely)	“Take into account”	Normative	“Take into account”	(unlikely)
Art. 10: Environmental targets and associated indicators	on basis of Art. 8 and 9 output (**)	Potentially all, they are “established so as to (...) guide progress towards achieving good environmental status”.				

* Likely to be progressively adapted to reflect a normative approach following implementation of Article 9

** Taking account of needs of the program of measure

Table 1.3. MSFD articles and additional requirements as a basis for indicators across the DPSIR spectrum (adapted from [VER 11])

Unlike the WFD, the MSFD regulatory framework does not include a specific aggregation algorithm for a compound judgment of overall environmental quality. So far, few methodologies exist to integrate all the elements into a single evaluation of a water body [BOR 08]. A model representation of ecosystem structure, functioning and processes seems to be necessary as a background canvas to allow a systemic approach to such integration [VER 11].

In the marine environmental policy context, the notion of an “ecosystem approach” is synonymous with “ecosystem approach to the management of human activities”, stressing that it is the latter that are being managed, not the environment as such. Against this background and of MSFD information needs, monitoring and assessment play a vital role. In this context, monitoring programs are embedded in Member States’ marine strategies, linking in with the cycle of monitoring and assessment activities (which are often managed regionally) and that of policy making and effectiveness evaluation [VER 11]. With a strong focus on environmental status defined by the MSFD, the monitoring programs will go beyond environmental monitoring to the extent that indicators require other types of data such as socioeconomic data. As a framework directive, and according to the subsidiarity principle, the MSFD does not automatically “force” the Member States to monitor any given substance or compound. There is no straightforward “European obligation” for any of the monitoring parameters.

The MSFD coverage of chemical water quality cannot be completely dissociated from that of the WFD, even if the latter concerns surface waters that extend to 1 mile of the coast. In proposing the MSFD, the European Commission had stressed the possible synergies of surface water management under the two regimes. The WFD approach to chemical quality is based on reaching water quality objectives based on EQS for a common list of priority substances and of priority hazardous substances [EUR 03, EUR 09b]. The MSFD further complements it by an approach based on the identification, as an element of environmental status, of a risk of possible “pollution effects” by substances in Member States wider

marine waters [VER 11]. In other words, “good environmental status” is linked with the absence of pollution effects as defined by the MSFD. Another emphasis is put on ensuring that all biota that we harvest as seafood are safe to eat. In a first phase, considering that the WFD implementation is more advanced in time (as it started in 2008) than that of the MSFD, Member States cover the management of most of the risks related to chemical substances – most of which originate from land-based sources – under the WFD river basin management plans (which include coastal waters, if not the territorial waters of the sea), and complement this with a review of any additional needs based on elements of information derived from their initial assessment of the environmental status (including risks and threats) of their marine waters [VER 11]. Novel elements regarding chemical quality are the inclusion, in MSFD Annex III, of parameters reflecting ocean acidification (pH, pCO₂), which is likely to be a basin-wide phenomenon best addressed at that level.

1.5. Science–policy interactions

1.5.1. *Scientific foundation of environmental policies: where do we stand?*

The need to strengthen links among scientific outputs and policy-making activities is subject to on-going debates and specific discussions in the water and marine sectors have examined concrete developments [QUE 10]. They tend to show that a conceptual framework for a science–policy interface among scientists, policymakers and stakeholders is required *inter alia* in the water and marine sectors. This section takes over general considerations about science and policy interfacing needs (after [QUE 07]). In a first instance, let us recall that key steps of the “environmental (including marine) policy chain” related to protection against pollution are based upon a scientific foundation and basic technical knowledge; these steps can be summarized as follows:

- describe what you want to protect;
- measure or describe status;

- define the level of protection according to well-defined objectives;
- identify pressures;
- quantify the relationship between pressure and environmental response;
- quantify the relationship between social and economic cost and pressure;
- identify the least-cost pathway;
- define the policy instrument;
- implement the policy instrument and assess response;
- take appropriate measures (control, remediation);
- review policy on the basis of scientific/technological progress.

The reliability of the overall chain will depend upon the effectiveness of the integration of scientific and technological knowledge in a timely fashion at each step of policy development, implementation and review. The knowledge of “environmental interfaces”, e.g. sediment–water interactions, and pollutant pathways at this interface (mobility, bioavailability, etc.) represents a basic feature for understanding the impacts of anthropogenic pressures on various (marine) environmental compartments. Hence, it has a direct impact on the way policy and related monitoring are designed, developed and implemented. This knowledge should, in principle, be tackled in a “holistic” fashion. In other words, it is difficult to understand the overall impact of a specific pressure on the environment by looking at only one compartment [QUE 07]. The different pollution pathways depend upon the nature of the pollutants (type and origin of chemical substances) and a high variety of environmental factors, such as the climate, hydrology (water flows and related sedimentation rates), geology, hydromorphology, physicochemical conditions (e.g. pH and redox potential) and biological interactions. In this respect, it is hard to understand a given pathway by looking at a single environmental compartment and through one discipline only.

To date, the knowledge of environmental interfaces is still limited by the lack of sufficient multidisciplinary studies. The progress is on-going but the scientific foundation is not considered to be sufficiently developed to be able to effectively assess the effectiveness of environmental policies in a holistic context. It is worth mentioning that, among research projects and related on-going activities, gathering of an increasing number of monitoring data (linked to EU policies and/or international programs such as the European Environment Agency's State-of-the-Environment program) and the development of models now provide a much better vision of the problems to be tackled and of the way to approach them. On the medium term (5-year horizon), it will be possible to establish a much better "holistic" evaluation of environmental interfaces and related pollution pathways. This will obviously have a direct effect on the implementation and review of related EU policies. In the longer term (10–15 years), the increasing number (and quality) of environmental databases, models and other monitoring facilities (e.g. Global Monitoring for Environment and Security) should enable us to look at the environment as a single entity instead of series of separate compartments.

1.5.2. EU scientific framework in support of water and marine policies

The treaty establishing the EU indicates that research framework programmes have to serve two main strategic objectives. First, to provide a scientific and technological basis for industry and encourage its international competitiveness. And second, to promote research activities in support of other EU policies. To this end, framework programmes (FPs) are designed to help solve problems and respond to major socioeconomic challenges faced by society. The research framework programme is the main instrument of EU for funding research and development. In this context, the European Commission has been supporting water and marine research through its successive FPs for research and technological development (RTD). The FP aims to foster scientific excellence, competitiveness and innovation through the promotion of better cooperation and coordination. It also aims to produce advances in knowledge and understanding, and to support the

implementation of related European policies. The FP is implemented through open “calls for proposals” and successful projects are selected after an evaluation procedure carried out with the help of external independent experts.

The Seventh Framework Programme covered priority areas reflecting EU research needs in sectors such as health, food and agriculture, information and communication technologies, nanosciences, energy, transport, socioeconomic sciences, space and security. Environment and climate change was one of these 10 priorities. It focused on knowledge of the interactions between the biosphere, ecosystems and human activities, and the development of new technologies, tools and services, with emphasis on the following issues:

- improved understanding and prediction of climate, earth and ocean systems changes;
- tools for monitoring, prevention and mitigation of environmental pressures and risks;
- management and conservation of natural resources.

More specifically, the research areas addressed pressures on environment and climate, impacts and feedback, environment and health, conservation and sustainable management of natural resources (including groundwater), evolution of marine environments, environmental technologies, understanding and prevention of natural hazards, forecasting methods and assessment tools, and earth observation.

Horizon 2020 has succeeded to the 7th Framework Programme; it is the biggest EU research program ever with some €79 billion of funding available over 7 years (2014–2020). By coupling research and innovation, the program seeks to help achieve excellent science, industrial leadership and tackle societal challenges. EU research funding has already brought together scientists and industry in Europe and from around the world to find solutions to a huge array of challenges, including environment protection.

1.5.3. Identification of research needs in the water policy sectors

It is not always possible to clearly establish the border between “basic” and “applied” research. Also the timing aspect (short-, medium- and long-term) is intimately linked to the way research instruments are being operated. The identification of research needs is of course fed by advances in scientific knowledge, but is also directly influenced by the evolution and requirements of policies. The needs for ensuring coincidence of research and policy agendas may depend upon the stage of development of the policy in a given thematic area. In this respect, one may distinguish three different categories of needs in the water policy sector, depending on timing considerations:

– *Short-term* (~1–2 years): Needs are basically concerning accessibility of research knowledge required for the development of policies on a short-term basis. Timing is not adapted to develop new types of research (unless very specific needs are identified, which may be sorted out in a 6–12 month period). Policy development also requires efficient and user-friendly access to background scientific information and archives; a typical example is the thematic strategies covered by the 6th Environment Action Programme (EAP). In this context, the time needed for the design, approval and operation of *ad hoc* calls for proposals makes it difficult to respond to short-term research needs, i.e. a specific research need expressed at a given time will rarely be met through a project selected under a call for proposals the year after. Therefore, to date such needs may only be tackled through Joint Research Centre (JRC) action lines (see section 2.2.3) that are identified in their annual work program and agreed by Environment Directorate-General, as well as through possible national research programs; successful examples exist in the sector of water policies. In the future, short-term needs could also be partially fulfilled through a coordination of national research calls for proposals (European Research Area-Network (ERA-NET) scheme).

– *Medium-term* (~2–5 years): The timing of medium-term research is adapted to responses to needs expressed in relation to the implementation agenda of well-defined policies (representing a

“stable platform” for building strong partnerships among policy implementers, the scientific community and various stakeholders). This is the case of the WFD, in support of which research activities have been carried out since the time of its adoption (2000) in response to needs linked to, for example analysis of pressures and impacts, characterization of water bodies (2004–2005) and economic analysis (2005). For the forthcoming milestones, the formulation of medium-term research needs will have to take into account, for example monitoring (2006) and the preparation phases (2007–2008) of the first river basin management plan to be published in 2009. The SSP mechanism (research in support of policies) within FP6 was well adapted to respond to such identified needs, i.e. a detailed description of research needs by policymakers and the follow-up of projects in close coordination with the scientific community represent key elements for achieving successful use and application of research to the policy making process. RTD projects running over a 2–3-year period may also fulfill medium-term research needs.

– *Long-term* (~5–10 years): Scientific progress in this respect supports either policy milestones, which are clearly identified at the 10-year horizon, or the legislation review process. In the case of the WFD, long-term research needs may be linked to the development of the program of measures, which has to be operational in 2012. It may also concern the review process of the technical requirements detailed in the relevant annexes of the directive, which should be known at the time of the Second River Basin Management Plan in 2015. It is expected that research activities, as they are developed under integrated projects (funded under FP6 or FP7), may respond to either well-defined milestones of the thematic policies or legislation review.

1.5.4. Interactions with the scientific community

At the start of projects which have been identified as relevant to water policies, there is certainly a need to clarify policy issues by describing the aims, milestones, and technical challenges to the RTD

project coordinators so that they understand the policy expectations over the duration of their project. These exchanges of information/knowledge rarely occur, which may lead to divergent directions being taken by the projects in comparison to policy orientations.

1.5.4.1. *Synthesis needs*

At the end of the projects, the most critical issue is the way the scientific information is “digested” so that it may be efficiently disseminated to policy endusers and possibly applied. This integration phase is certainly the weakest link in the science–policy chain. Indeed, only a small percentage of RTD projects are known to policy implementers, which illustrates the need to improve awareness about RTD outputs and also to encourage policy actors to reflect on research needs linked to their portfolios. This may be translated into needs to carry out synthesis works in the form of “policy digests” (addressed to the scientific community from the policy implementer’s side) and “science digests” (prepared by the scientific community for the policy implementers).

1.5.4.2. *Exchange platforms*

As a follow-up to RTD or financial instrument for the environment (LIFE) projects, useful interactions may occur at the occasion of yearly meetings. Participation by policy officers in all project meetings may not be practicable due to a lack of resources but efforts are needed to organize regular joint meetings focusing on specific themes. This is already taking place in the WFD sector [QUE 05] and should be systematized.

1.5.4.3. *Toward a “science–policy interface”*

As discussed in section 4.2, at the present stage efforts to present results and demonstrate projects are lacking in a form that policymakers may easily use, e.g. “science-digested” policy briefs. On the reverse side, the consideration of research results by the policy making community is not straightforward, mainly for political reasons and due to difficulties integrating the latest research developments in

legislation. The difficulty is enhanced by the fact that the policy making community is probably not defining its role as “client” sufficiently well. In other words, the dialogue and communication are far from being what one would hope to ensure an efficient flow of information. In this respect, improvements could be achieved through the development of a “science–policy interface” based on a coordination of relevant programs/projects with direct relevance to the WFD implementation [QUE 05]. In other words, strategies should identify needs for short-, medium- and long-term scientific developments and should establish an interface so that R&D results are synthesized in a way that can efficiently feed the implementation and further reviews of the policies. This interface should include the following:

- A screening phase evaluating which type of research is needed (background information or tailor-made research and demonstration) in accordance with the policy step of concern (e.g. development of the daughter directives covered by the WFD, implementation issues, reviewing). This is already happening through regular contact within commission services and with the scientific community.

- A mechanism to ensure that the most promising research projects in support of the policies are “validated” through demonstration activities, disseminated efficiently, and applied at the appropriate level (regional, national or EU). This is not yet or is rarely the case, but there are increasing examples of RTD projects that include a demonstration phase.

- A management scheme involving both scientists and policy makers to discuss the corresponding research and policy agendas from the very beginning in order to ensure a more structured communication at all appropriate levels of policy formulation, development, implementation and review. This is hardly operational to date.

More than dissemination and application, the interface should establish strong links between the different funding mechanisms existing at the EU level and the thematic policies. This should enable us to promote pilot projects combining the implementation of the results of successfully completed EU-funded RTD or demonstration

projects with the implementation of related policies. This would allow the formation of new and innovative partnerships by combining various EU (RTD, LIFE, Cooperation in Science and Technology (COST), structural and cohesion funds (Interreg projects), agricultural funds, etc.) and regional/national funding mechanisms, and the establishment of a collaborative partnership involving scientists, policy makers, managers and other stakeholders for the effective integration of scientific outputs in policy and management decisions. At present, however, such coordination is not operational.

1.5.5. Science-based development of an integrated environmental policy

In the first instance, we may ask the basic question: is our scientific (multidisciplinary) knowledge sufficient to develop a more integrated policy? The on-going discussions show that the scientific base is likely still not sufficiently consolidated at this stage but that a tight coordination mechanism and tailor-made developments in FP7 could lead to the establishment of an operational science–policy interfacing mechanism on the 2015–2020 horizon.

Noteworthy is the consideration about scientific uncertainty for which awareness is raising. Such considerations seek to invoke the standard of evidence that “guilt” must be demonstrated “beyond a reasonable doubt”. However, given the complexity of environmental pollution pathways, this would mean that the reality of environmental risks would not be accepted until something had actually happened. This is against the prevention principle and is not acceptable. In the light of the precautionary principle, however, where there are threats of serious or irreversible environmental damage, lack of full scientific certainty should not be used as a reason for postponing measures aimed at preventing environmental degradation.

The WFD is a good example of such an evolution tending to better policy integration. This is illustrated in Figure 1.2, showing a progressive integration of existing directives that will be repealed under the WFD.

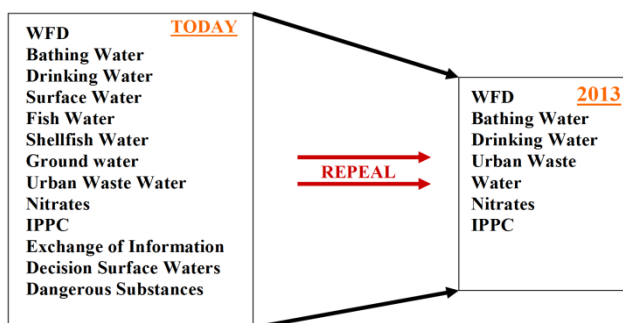


Figure 1.2. Policy integration trends under the WFD

In the context of WFD requirements, the following observations can be made:

- Monitoring and data reporting of environmental status and trends need to be coordinated at EU level in the framework of a common mechanism. This is the goal of the Water Information System for Europe (WISE [DEU 06]), which allowed a considerable step forward at the 2008–2009 horizon. Coordinated reporting and data sharing should constitute the core basis for water policy implementation and review within the next decade. In the light of increasing considerations about climate change (and its impact on water management), we may wonder whether data sharing should not be expanded at a global scale in the form of a global WISE to be closely linked to Global Monitoring for Environment and Security developments.

- Building up environmental databases, such as the one to be developed under the WFD monitoring programs, should enable us to test/validate existing models and develop new models better able to evaluate environmental risks linked to pollution pathways, and thus better evaluate the efficiency of policy responses. This is closely linked to the knowledge-based considerations stated in this chapter.

- Risk assessments and programs of measures need to be coordinated in the light of effective implementation of directives in force (namely all directives listed in Annex VI, part A, of the WFD).

The consequence of better integration of scientific knowledge and policies might result in a few pieces of framework legislation in the long-term. In Figure 1.2, RTD projects studying “environmental interfaces” are closely interlinked to specific policies. The overall cycle, however, could be conceived in one single environmental circle. In this context, a better – knowledge-based – appraisal of risks in the context of concerted planning (e.g. at river basin level) would facilitate the design of monitoring programs (avoiding duplication, focusing on specific features) and the elaboration of programs of measures. The way framework directives are being developed opens the door for increasing integration, which should be pursued and linked to a sound and validated scientific foundation.

1.6. Conclusions

In this chapter, we may be puzzled by the high number of international organizations, national, international, regional and sometimes global conventions, which all deal with the study (primarily the monitoring) of chemicals in the marine environment [KRA 01, ROO 10, ROO 11]. Some of these programs have been running for many years or even decades, principally within the industrialized northern hemisphere. The output of these programs has been used to identify areas or regions of concern, estimate the hazards caused by chemicals to human beings and the marine environment and assess the effectiveness of the measures taken. Over the past 40 years, the objectives of monitoring harmful substances in fish and shellfish as potential hazards to human health have changed into an ecosystem approach where – in Europe under the WFD and MSFD – good environmental status will be reached. Monitoring is one of the management tools used for assessing the quality status and the temporal trends, i.e. as a policy tool. These long-term monitoring programs are expected and able to demonstrate changes in the levels of chemicals in the marine environment resulting from policy actions [ROO 11a]. Despite this, the present situation is far from ideal. Collecting and reporting of the data is often incomplete, which hampers the assessment process, and this particular aspect deserves special attention at all the levels. Clearly, it is one thing to

conceive a monitoring program, it is quite another to implement it. This requires time and effort spent by all parties involved and we should not forget that monitoring of the marine environment is a time-consuming and expensive process [ROO 11a]. A further fundamental difficulty for an efficient interface between research and policy arises from the fact that research and policy have different and varying agendas [ROO 11b]. Although policy tends to focus on the short-term perspective, science envisages a long-term perspective. Moreover, while policy tries to involve the development of acceptable compromises, the scientific community aims to work toward the collection of objective scientific facts and the development of reasonable theories. This ambiguity is also present in the current monitoring programs. There is a clear need for more data, both in terms of quantity (e.g. spatial and temporal distribution of data points) and quality (e.g. number of chemicals determined) if we are to understand the status of the marine environment. Given the natural variability, fewer data are often disastrous for a proper assessment, whereas more data imply more resources, which inevitably meet restraints. Nevertheless, we can wonder if the potential consequences of limited data sets outweigh the costs of obtaining the right information [ROO 11].

An important aspect is that there is a general awareness that the data, which are produced and stored, should be of high and, specifically, well-defined quality. The emphasis on the awareness of quality assurance and quality control (QA/QC) is one of the major achievements of chemical monitoring to date. Successful intercomparison exercises and programs such as QUASIMEME ([TOP 97, WEL 00, WEL 06]; www.quasimeme.org) have highlighted the need for rigorous QA/QC in chemical monitoring. QA/QC is now omnipresent and an essential element of all well-defined monitoring programs. Among other things, this implies the obligation of laboratories to participate in international intercomparison exercises or proficiency testing schemes and to have documented QA/QC procedures if not outright accreditation. This is a part of the overall metrological framework of this book, which is discussed in Chapter 2.

Monitoring and Quality Assurance¹

2.1. Monitoring of what?

2.1.1. Selection of compartments

Kramer [KRA 11] provides a historical perspective of marine monitoring, whose basics will be discussed in this chapter. He recalls that when monitoring started, fish and shellfish (biological tissue) were the matrix of interest as the focus was on potential hazards to human health from the consumption of seafood. This was the case of the initial North Sea, Baltic and North Atlantic baseline studies in the early 1970s. The concept of “mussel watch” was presented by Goldberg [GOL 75] as a method to assess the health of the ocean [KRA 11], which is based on the capacity of mussel species (in particular *Mytilus edulis*) to concentrate many pollutants in their tissues, hence enabling us to use them for (annual) trend monitoring of concentrations of, e.g., halogenated carbons, transuranics, heavy metals and petroleum [GOL 75]. This approach has been used by the

¹ The following abbreviations are used in the chapter: NIST: USA National Institute of Standards and Technology; IAEA: International Atomic Energy Agency; BCR: EC Bureau of Community Reference, now EC–Joint Research Center–Institute for Reference Materials and Measurements (EC–JRC–IRMM); NRC: Canada National Research Council; NWRI: National Water Research Institute, Environment Canada; CIL: Cambridge Isotope Laboratories, USA; NIES: National Institute for Environmental Standards, Environment Agency, Japan; LGC: Laboratory of the Government Chemist; RTC: RT Corporation (USA); NRCCRM: National Research Center for CRMs (China).

US NOAA National Status and Trends Programme and in many other countries around the world [OCO 94]. Concentrations of trace metals and other pollutants, such as polycyclic aromatic hydrocarbons (PAHs) and PCBs, are routinely monitored in the OSPAR and HELCOM areas [KRA 11]. In the water compartment, trace metals (since the late 1970s) and nutrients (from 1990 onwards) have been routinely monitored under ICES and HELCOM programs. The OSPARCOM JMP initiated sediment monitoring in 1982. The hydrophobic characters of many organic pollutants mean that they are predominantly present in sediment and suspended particulate matter, thus justifying this matrix as a compartment of choice for trend monitoring. This has been recognized by the WFD amendment EQS Directive, for example, which states that hydrophobic compounds may be monitored in water and/or biota (instead of water).

2.1.2. Selection of compounds

At the end of the 1960s, chemical substances to be monitored were selected on the basis of their human toxicity, aquatic toxicity and aesthetic effect [GES 69]. This was reviewed at a later stage, when harmful substances that may have deleterious effects on human health and economic and cultural activities in the marine environment and coastal areas were added, and consideration given with regard to (harm to) living resources in the sea as a major source of protein-rich food [GES 76]. Restrictive or preventive measures were recommended for important pollutants issued from domestic sewage, pulp and paper mill wastes, organochlorine pesticides, PCBs, mercury and its compounds, organolead compounds, elemental phosphorus, silver and several organic chemicals, often solvents [KRA 11]. With its background in fisheries research, ICES initially limited its monitoring activities to toxicants in fish and shellfish, focusing in particular on heavy metals (Cd and Hg), selected organochlorine pesticides and PCBs accumulated in a few indicator species such as mussels, herring, cod and plaice. An increased number of substances were considered for the North Atlantic baseline study as well as investigations of the Baltic Sea [ICE 74], namely organohalogen compounds (hexachlorobenzene (HCB), PCBs, pesticides such as DDTs, dieldrin, chlordane and hexachlorocyclohexane (HCH)

isomers), metals including Hg, Pb, Cu, Cd, Cr (replaced by As for the Baltic) and Zn, petroleum hydrocarbons and nutrients (nitrogen compounds and total phosphorus). This list formed the basis for the HELCOM's pilot phase baseline monitoring program [KRA 11]. The second phase listed, next to basic oceanographic variables, nutrients, heavy metals and petroleum and chlorinated hydrocarbons in seawater, and heavy metals and chlorinated hydrocarbons in fish and shellfish [HEL 88]. Gradually, as more chemicals were identified as being harmful to the (marine) environment, an increased number of chemical compounds were added to routine monitoring programs, e.g. more pesticides, organotin compounds (such as tributyltin (TBT), which affects oysters and marine gastropods) and brominated compounds which are used as flame retardants [KRA 11].

The EU Directive on Priority Substances (setting Environmental Quality Standards) is a daughter directive to the WFD, hence applying to Member States' coastal waters. It defines the limits on concentrations of 33 priority substances and nine other pollutants in community surface waters, including the coastal waters [EUR 08a]. The list includes trace metals (Cd, Hg, Ni, Pb and TBT), different pesticide groups, seven PAHs, (chlorinated) solvents and other compounds such as nonylphenol and brominated diphenylether.

Focusing solely on the marine environment, the list of OSPAR priority groups of chemicals includes trace metals (Cd, Pb and Hg), organometallic compounds (of Pb, Hg and Sn), organohalogens (including short-chained chlorinated paraffins, perfluorooctane sulfonates, polychlorinated dibenzodioxins ((PCDDs) and polychlorinated dibenzofurans (PCDFs)), PCBs, brominated flame retardants and other polybrominated diphenyl ethers (PBDEs), hexabromocyclododecane and tetrabromobisphenol-A. Furthermore, seven pesticides are included, such as endosulfan, HCH isomers (including lindane), pentachlorophenol and trifluralin. Other groups include phenols, phthalates (such as DBP and bis(2-ethylhexyl) phthalate (DEHP)), PAHs, pharmaceuticals and personal care and other substances [OSP 10]. Monitoring of nutrients

started as a result of decisions to reduce nutrient inputs in the OSPAR area by 50% [DE 86].

2.2. Quality of data

2.2.1. Introduction

The awareness of the quality of data from marine monitoring programs became an important issue from the early 1970s onward, when it became obvious that data produced by different laboratories often did not match [KRA 11]. Discrepancies were investigated through interlaboratory comparisons, which showed that, for example, the results of seawater analyses for trace metals differed by two and even three orders of magnitude between different laboratories. Goldberg and Taylor [GOL 85] argued that only validated data should be included in databases in order to ensure that only quality data are used for decision-making and mapping purposes. Batley [BAL 99] stressed that monitoring data should not be published without demonstrable evidence of quality practices in all aspects of the monitoring exercises [BAL 99].

The design of several instruments was elaborated and developed to improve the quality of monitoring data from the early 1970s [KRA 11], including laboratories' QA measures and the building up of continuous analytical quality control program via participation in interlaboratory comparison studies, the development and implementation of guidelines on sampling, analysis and QA/QC, the implementation of QC by the use of certified reference materials (CRMs) and QC materials in the analytical process, and the participation in laboratory performance studies (proficiency testing). Within laboratory QC and between laboratory QC had to be demonstrated throughout the monitoring program [NIC 89].

European policies also recognized the need for quality data from monitoring programs, namely through the European Commission Directive 2009/90/EC [EUR 09], which lays down technical specifications for chemical analysis and monitoring of water carried

out in the WFD framework. This regulation establishes minimum performance criteria for methods of analysis to be applied by Member States when monitoring water status, sediment and biota, as well as rules for demonstrating the quality of analytical setting quality criteria for other directives dealing with monitoring [KRA 11, LEP 09]. It explicitly addresses QA and QC, and makes it mandatory that laboratories demonstrate their competence by participation in proficiency testing programs and analysis of representative reference materials.

2.2.2. Interlaboratory comparisons

The starting point of interlaboratory comparison studies was to demonstrate that sufficient agreement was achieved among different laboratories' results to compare monitoring data from different countries [KRA 11]. Initial studies showed that for the majority of analytes and matrices, analytical results were far from being in good agreement, and hence efforts were deemed necessary to improve this situation. Interlaboratory comparisons thus started to become a training tool for improvement of laboratory skills [KRA 11].

The first ICES intercomparison was carried out in 1972 on biological tissue analyses for Hg and chlorinated hydrocarbons, following the development of the compounds and matrices that were monitored in the 1970s–1990s period. It was followed (for biological tissues) by a total of nine exercises for trace metals (1972–1989), three for hydrocarbons (1984–1990) and no less than 15 for organochlorine compounds (1972–1993). Seawater analyses were then tested through 11 interlaboratory comparisons on trace metal analysis (1976–1996), and five studies for nutrients (1989 and 1993) ([ICE 92] quoted by [KRA 11]). Sediment intercomparison studies started in 1980, focusing on hydrocarbons (1980) and chlorinated hydrocarbons (1980 and 1993), and then on heavy metals with a total of nine studies (1983–1993). The role of (ICES) interlaboratory comparisons has been gradually taken over by participation in proficiency tests, such as

the QUASIMEME program (see section 2.2.6), that was a combined ICES/QUASIMEME exercise [KRA 11].

A feature which was recognized as being very useful was the exchange of information during meetings where the outcome of the interlaboratory trials was discussed, since experts could exchange views and discuss how to improve their methods in a collegial atmosphere. Comparisons were often based on coefficient of variations (CVs); the lower the concentration, the higher the variance in the analytical results as postulated by Horwitz [HOR 82]. Today, the variance (CV) between laboratories for groups of analytes is as follows: $CV(\text{nutrients}) < CV(\text{trace elements}) < CV(\text{chlorinated hydrocarbons})$ [KRA 11].

On several occasions, it proved too difficult to start with “real” matrices since analytical results varied too much; this was the case, for example, with chlorinated organic compounds in biological tissue [KRA 11]. In these cases, a stepwise approach was initiated, starting on a less complex analysis level, e.g. calibration solutions and/or extracts that were centrally prepared. Natural matrices were only reconsidered in the interlaboratory tests when harmonization could be achieved for these samples.

Besides ICES (and HELCOM), the other organization coordinating interlaboratory comparisons in the marine sector was the International Atomic Energy Agency (IAEA), which was used initially for the analysis of radionuclides, then for many other organic and inorganic compounds. Interlaboratory trials were carried out in the context of the World Health Organization/FAO/UNEP Joint Project MEDPOL (see Chapter 1), which has been the cornerstone of the Mediterranean action plan and has been instrumental in developing the capabilities of (Mediterranean) countries to measure and assess marine pollution [CAR 01]. The IAEA Marine Environment Laboratory in Monaco acted as an analytical and training center for MEDPOL.

Since the implementation of QA/QC procedures in analytical laboratory activities, the comparability of data has improved

considerably. Although intercomparisons were not only devoted to the analysis itself, and also included, for example, for trace metals sampling [BEW 82] and filtration procedures [BEW 85], Kramer [KRA 94] argued that most QA/QC procedures were limited to the activities within the laboratory, and that they should be extended to the activities before samples reached the laboratory, such as sampling, sample treatment and storage [KRA 11]. This can be achieved by using written standard operating procedures (SOPs), agreeing on a specifically developed sampling plan, and safeguarding full documentation at every stage of the operation, including unexpected events [WAG 95]. Davies and Wells [DAV 97] concluded that QA and the interpretation of international marine monitoring programs had improved considerably, in part as a result of the series of interlaboratory comparison studies and laboratory performance studies. There still was – and is – an urgent need for CRMs, and QCMs as instruments for further improvement of the analytical quality.

2.2.3. Guidelines

The need for national guidelines and SOPs were highlighted when it became clear that there was a poor overlap in monitoring results. Without trying to be complete, few organizations produced guidelines about sampling and analyzed them as discussed below [KRA 11]. ICES developed a series of publications about Techniques in Marine Environmental Sciences (TIMES) from 1987 onward, on sampling and storage methods for trace metals in seawater [YEA 87], good laboratory practice and QA [VIJ 87] or the review of methods for analysis of hydrocarbons in seawater, biota, and sediments [HER 91]. OSPAR also produced monitoring guidelines when the JAMP activities (see Chapter 1) were initiated [KRA 11]. These guidelines are regularly reviewed in collaboration with ICES and, where necessary, updated to take into account new developments, including the inclusion of new monitoring parameters (latest updated versions are electronically available at www.OSPAR.org). Similarly, HELCOM has published guidelines in support of its monitoring

program [HEL 88], e.g. the “General guidelines on QA for monitoring in the Baltic Sea” (www.helcom.fi). Finally, the UNEP Regional Seas Programme was also supported by the publication of guidelines dealing with the development and testing of reference methods, the preparation of reference materials, QC of monitoring data, in the organization of intercalibration exercises, and in QA/QC training [IBE 95].

2.2.4. (Certified) reference materials

The production and use of (certified) reference materials in the marine monitoring field are described in detail in section 2.3. This chapter provides some details about historical features and producers described by Kramer [KRA 11]. What is probably the first “standard seawater” material has been produced since the early 20th century and used as a primary standard for the calibration of chlorinity/salinity measurements [CUL 78].

The needs for reference materials coincided with the development of pollution monitoring programs. The National Research Council Canada (NRCC) was the first organization recognizing the need for a series of environmental CRMs to answer the increasing needs of monitoring programs [KRA 11], e.g. marine sediments, seawater and biological tissues (lobster) certified for trace metals [WAL 87].

In Europe, the European Commissions’ Community Bureau of Reference (BCR) was established in 1973 [VAN 77] and existed until 2003, when its activities were transferred to the Institute for Reference Materials and Measurements, one of the JRC institutes of the European Commission [QUE 03] and BCR remained only a brand name. The BCR also recognized reference material needs in support of marine monitoring activities and started CRM production (see Chapter 3), e.g. for trace metals in sea lettuce, estuarine sediment, mussel tissue and seawater, and for PCBs in cod liver oil, mussel tissue, etc. Production needs followed the trends in policy and monitoring practices, where more (and more complex) hazardous substances had to be analyzed, which resulted in the production of

new CRMs for the analysis of methylmercury in tuna, TBT, and other organotin compounds in mussel tissue and coastal sediment, etc.

Another international producer is the Japanese National Institute for Environmental Studies (NIES), which produced CRMs of mussel tissue and Sargasso seaweed for trace element analyses. The IAEA also produced marine reference materials in its Marine Environment Laboratory (Monaco).

The US National Institute of Standards and Technology (NIST) has been a major worldwide producer of environmental matrix CRMs since the 1990s [KRA 11]. In recent years, the organization initiated a new type of production of matrix materials certified for a wide range of analytes, thus serving the needs of laboratories that have to respond to the monitoring requirements, such as the US EPA. Examples are given in Chapter 3.

Several overviews of available marine CRMs have been produced (e.g. [CAN 92, KRA 98, PEL 11]); Chapter 3 provides a snapshot of existing materials. Today web-based databases are readily accessible, such as the Virtual Institute for Reference Materials (www.VIRM.net). Unfortunately, not all analytes in all matrices requested by monitoring programs are fully covered. Notably, reference materials for organics in water are still lacking due to problems in production (stability of the analytes) [WEL 98].

2.2.5. Laboratory performance studies

Laboratory performance studies, also known as proficiency tests (PTs), aim to verify the capability of laboratories to analyze a given analyte in a given matrix with a sufficiently high accuracy and precision. This is usually coordinated by a central organization, which sends samples with unknown composition to a large group of laboratories [KRA 11]. Their analytical results are intercompared in the light of a reference value and (accepted) deviation unit, and a measure of their proficiency (usually as a z-score) is confidentially reported back to the laboratory [THO 06]. A framework for PTs is

guided by the ISO/IEC 17043 standard [ISO 10], which replaced ISO/IEC Guide 43-1:1997.

In the early 1990, doubts about the quality of data were expressed by the JMG of OSPARCOM, and experts highlighted the need to undertake measures to guarantee a comparability of monitoring data among different countries in the context of the preparation of the 1993 Quality Status Report of the NSTF [KRA 11]. This need was also supported by other international programs conducting similar monitoring programs in other regional seas [KUL 86, TOP 92]. In view of developing a collaborative approach to serve these monitoring programs in the (further) development of their QA and internal and external QC, the European Community funded the Quality Assurance in Marine Monitoring in Europe (QUASIMEME) project, which started in 1992 [WEL 93]. This program has been instrumental in the development of laboratory performance studies in support of monitoring of the (initially European) marine environment [KRA 11]. A wide range of PTs has been carried out, including PTs on nutrients in seawater, trace metals, PCBs and organochlorine pesticides in sediment and biological tissue, and PAHs in sediment (extract), thus covering nearly all analytes and matrices of monitoring interest [WEL 97, TOP 97]. QUASIMEME became a self-sustained PT program after the end of the EU-funded project, and it is still continuing with the regular production of reference materials and organization of PT schemes in support of marine monitoring programs. Other international organizations active in laboratory performance studies include the IAEA for organic contaminants (PCBs and sterols) and trace metals, often including methylmercury [DE 07].

Nowadays, laboratories have to be accredited and demonstrate their competence by meeting internal and external QA and QC measures, and complying with the ISO 17025 standard [ISO05]. All institutes/ laboratories submitting data to OSPAR or HELCOM databases have to participate in regular intercomparison exercises and PT schemes arranged, e.g. under QUASIMEME, ICES, HELCOM and IAEA, using CRMs and QC materials on a regular basis [KRA 11].

2.2.6. Example: monitoring of trace metals in seawater

Kramer [KRA 11] reports an example about monitoring of trace metals in seawater, which is illustrative of the QA/QC trends described in this chapter (Figure 2.1).

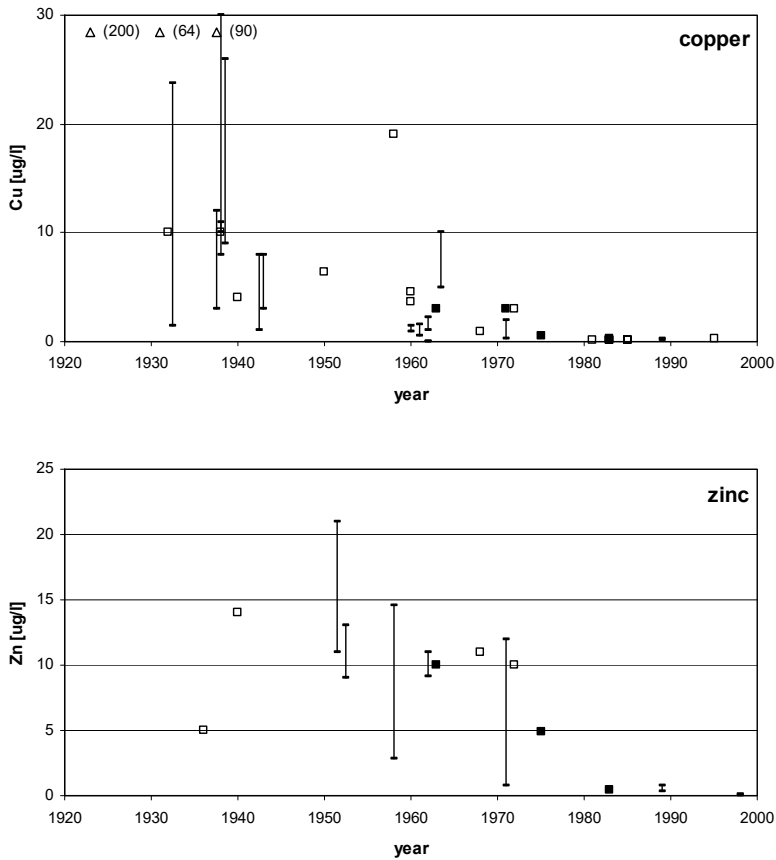


Figure 2.1. Concentration of dissolved copper (Cu) and zinc (Zn) reported for open seawaters since 1930s. This figure has been compiled by Kramer [KRA 11] on the basis of various authors

Reporting of trace metal concentrations in seawater started at the beginning of the 20th Century (As, Au, Cu, Fe, Hg) or even earlier (Ag, Zn), as reviewed by Johnston [JOH 65]; some data at the ng/L level, such as cadmium and lead, were reported as early as late 1950s [KRA 11]. At the time, sophisticated analytical methods were not available for measuring low metal concentrations, and the lack of attention given to contamination control and QA/QC concentrations resulted in a large spread and variation of results [KRA 11]. Analytical improvements enabled this spread to be reduced which resulted in decreasing concentrations as observed in temporal trends (and concentration ranges) for open seawaters, e.g. copper and zinc, reported since the 1930s with (very) high observations with a large spread in results within data series until the mid-1970s. It is only after awareness was raised about contamination risks (explaining high results), and risks during sampling, and about the need to implement QC procedures that the agreement among results became closer and the spread smaller [KRA 11]. Kramer [KRA 11] reports the “accepted” average concentrations for seawater, taken from reports published by Goldberg [GOL 65], Riley and Chester [RIL 71], Brewer [BRE 75] and Bruland [BRU 83] in Figure 2.1. The illustration clearly demonstrates advances in analytical chemistry in monitoring programs, showing that the large decreases in concentrations for open seawaters were not related to a decrease in pollution but rather to reduced contamination of samples during the analytical processes. The “typical” concentration values defined around 1980–1985 for the various trace elements in open seawater have hardly changed since [KRA 11]. From 1982 onward, improved analytical methods enabled the reporting of results with one decimal, and method performances have drastically improved since then, with the introduction of metal complexation/extraction coupled to graphite furnace atomic absorption spectrometry (GFAAS) in 1986, then inductively-coupled plasma ionization mass spectrometry (ICP-MS) after 2005 [KRA 11]. Analytical methods used for marine monitoring are presented in detail in Chapter 3.

From the earlier discussion, it can be concluded that early problems in the quality of the marine monitoring data have been

solved by interlaboratory comparisons and rigorous implementation of QA and QC programs [KRA 11]. One of the most critical aspects of QA/QC programs, which enabled these improvements, is the increased production of (certified) reference materials, which is discussed in the following section.

2.3. Certified reference materials

2.3.1. Introduction

QA/control principles are widely discussed in a book in the Water Quality Measurements Series [QUE 02a] and will not be repeated here. In this chapter, the focus is on CRMs, which are widely-recognized tools for the verification of the accuracy of analytical techniques [QUE 99c], forming an integral part of QC systems used in accreditation schemes. The availability of CRMs for the QC of marine chemical measurements in environmental matrices has drastically improved over the last three decades, and the materials cover a large range of matrices and parameters [ULB 06, WIS 06]. This chapter recalls general principles about the production and use of reference materials, and provides general information about matrix CRMs used in marine monitoring analyses. This is based on a review made by Pellizzato *et al.* [PEL 11], which includes (not exhaustive) tables listing materials that will not be repeated here (readers are invited to consult the publication in question for additional information).

2.3.2. Production and use of reference materials

2.3.2.1. Definitions

CRMs are also referred to as “standard reference materials” (SRMs) by the NIST, USA. They allow analytical laboratories to link their results with those of internationally-recognized standards. In addition, CRMs enable the user to verify his or her performance at

any desired moment in terms of accuracy. CRMs can be the following:

1) They can be pure substances or solutions to be used for calibration and/or identification; pure substances are usually certified by establishing the maximum amount, in mass fractions, of the impurities, which remain in the purified substance. For metals, it is possible to estimate the mass fraction of other elements within the limits of detection of the available analytical methods, typically some fractions of a percent. For inorganic salts or oxides, this estimate may be more difficult. The stoichiometry also has to be assessed (e.g. water of crystallization).

2) They can be materials of a known matrix composition for the calibration of a certain type of comparative measuring instrument certified calibration solutions have to be prepared on a mass basis within specially trained and skilled laboratories. Certification will be based on a metrologically-valid weighing procedure after a proper purity and stoichiometry assessment.

3) CRMs can be matrix reference materials that, as far as possible, represent the matrix being analyzed by the user and have a certified content. Such materials are mainly intended for the verification of a measurement process; matrix CRMs are composed of a natural unknown or only partially known matrix in which the amounts of a certain number of substances are certified. Usually, analysts prefer fully natural materials that are as similar as possible to real samples and not artificially enriched materials. In addition, spiking of solids with solutions containing the contaminants leads to unknown losses in the preparation. Therefore, gravimetric results can only rarely be used to certify the material. Certification can only be based on an analytical approach using complex procedures with limited precision compared to mass determinations.

4) They can be methodologically-defined reference materials; in such materials, the certified value is defined by the method applied following a very strict analytical protocol, e.g. the reference material may be certified following a standardized method (e.g. single or sequential extraction procedure).

CRMs are products of very high added value. Their production and certification is very costly. Therefore, except calibration materials for comparative methods, they should be reserved for selected verification of analytical procedures and not for daily checks, such as intralaboratory statistical control, or for interlaboratory studies (roundrobins).

2.3.2.2. Requirements for the preparation of reference materials

This section describes the general requirements that are applicable to all kinds of CRMs, including those used in marine monitoring. A series of requirements have to be respected for the preparation of reference materials and CRMs. They include three basic properties: (1) the representativity of the sample; (2) the homogeneity; and (3) the long-term stability. The preparation procedure has to be adapted to the type of material to be prepared and to the objective it has to fulfill.

In order to arrive at sound conclusions on the analytical performance of a method or of a laboratory, the reference material should be as similar as possible to samples currently analyzed by the laboratory in its daily practice. This means that the reference material should pose similar difficulties, i.e. induce the same sources of error that can be encountered in analyzing the real samples. Materials that would pose more difficulties than the daily samples may also fulfill the requested task of evaluating the method's or laboratory's performance as long as they do not require additional handling, which may induce large additional uncertainties.

The requirement of representativity of the reference material means in most cases similarity of:

- the matrix composition;
- the chemical form and concentrations of the analytes;
- the way of binding of these analytes;
- the fingerprint pattern of possible interferences;
- the physical status of the material.

In preparing a reference material, these items should be taken into full consideration. In many cases, and for practical reasons, the similarity cannot always be entirely achieved. The material has to be homogeneous and stable in order to ensure that samples delivered to the laboratories are the same and compromises often have to be made at the preparation stage. Certain parameters of interest that characterize the difficulty of some real natural samples may disappear, e.g. inhomogeneity of real samples may need a special technique or strategy of subsampling. Some other problems may arise or may be enhanced due to the treatment necessary to homogenize or stabilize the material. It is up to the producer and to the user to define the degree of acceptability of compromises. The user should be informed of the real status of the sample on the treatment performed and perhaps on the treatment to be applied to bring the sample back to a more representative form prior to the analysis.

The amount of material to be collected has to be adapted to the purpose of the analysis and is a function of the analytical sample size, stability, shelf-life, frequency of use and potential market. It is sometimes better to prepare a limited batch of samples so that the stock lasts a sufficient time and to prepare a new batch of material when new requirements for modern analytical techniques appear or when regulations have changed. The amount collected may vary from a few kilograms of solid material (e.g. sediments) or even grams (e.g. biota), from several liters (e.g. seawater) or milliliters (e.g. liver oil extracts) for the preparation of a reference material for the statistical control of a method, up to hundreds of kilograms of solid material or several cubic meters of water in the case of a reference material for large interlaboratory studies or CRM production. The producer needs to be equipped to treat the necessary amount of material without substantially changing the representativity. The treatment of 3–5 kg of raw material is the limit for usual laboratory equipment and manual processing; for larger batches, and especially larger volumes of material, it is necessary to scale up to half or even industrial size machines.

Typical operations for the preparation of a reference material are stabilization, crushing, grinding, sieving (for solid materials), filtering

(for liquid materials), mixing or homogenization of the material. They can only be performed in specialized laboratories or industries.

A very sensitive and difficult step in the preparation is the stabilization of the raw material, which may affect its representativity. Stabilization is necessary to guarantee that the materials remain unchanged over time. It has to be adapted to each particular case, with regard to the matrix and substance to be determined, and should be studied in detail before processing the batch of the reference material. Pure and synthetic mixtures of solid materials are usually stable and do not need a particular treatment. Natural products or synthetic liquid or gaseous mixtures are often highly unstable. Solid materials are often dried to avoid chemical or microbiological activity. This may be achieved by heat-drying (e.g. for sediments to be analyzed for trace elements) or by freeze-drying (e.g. plant or animal tissues, sediments, samples to be analyzed for trace organics, etc.), or by fixing the water within the material with chemical additives.

Some materials can be sterilized by gamma irradiation (^{60}Co source). In reality, this treatment is mainly possible for materials due to be used for element determinations, as many compounds decay upon gamma irradiation (e.g. tin compounds [QUE 94]). Sterilization by pasteurization or similar methods and addition of chemical preservatives are other alternatives. Stabilization by simple deepfreezing is also possible, but induces difficulties in transport and storage. In addition, the material can only be used once as unfreezing/refreezing may not lead to a homogeneous material.

The composition of the material and the parameters investigated should remain unchanged over the entire period of use of the material. The study of the material's stability over time will mainly depend on its role. If the material is foreseen to be used in a short-term interlaboratory study, the stability may only be monitored over the real duration of the exercise and additionally mimic situations that may be encountered during its short lifetime, e.g. transport under severe climatic conditions. This may vary from some hours (e.g. microbiological samples) to several years for a CRM. The (in)stability should be studied or known before the reference material is produced and should be monitored on the batch of reference material for its

whole lifetime. The stability can be estimated by evaluating the characteristics of the material under accelerated ageing conditions, e.g. elevated temperatures over long periods of time.

A material can only serve as a reference when at each occasion it is analyzed an identical portion is available. Therefore, when stabilized, the material must be homogenized to ensure sufficient within- and between-vial homogeneity for the property value to be certified. The inhomogeneity of the material should not significantly affect the total uncertainty of the measurement. Homogenization is not the most difficult problem for gases and liquids, despite the fact that some samples, e.g. estuarine water, may be prone to flocculation during the homogenization process [KRA 94]. Solid materials or any material composed of various phases (e.g. aerosols and suspensions) are obviously more difficult to homogenize or to keep homogenous. Experience shows that for small particle sizes, i.e. less than 125 μm , narrow particle size distribution and homogeneous density of the particles, a good homogeneity is achieved and can be maintained or reobtained even after long storage periods with simple shaking or mixing with laboratory tools. Unfortunately, the small particle size presents some drawbacks as it leads to materials that are usually easier to analyze than real samples (better extractability of analytes or easier matrix digestion because of the large contact surface).

The quantity of material in the bottle or ampoule should be sufficient to possibly perform several determinations. The more subsamples taken, the higher the risk that the remaining bulk is no longer identical to the initial material. In addition, the producer must guarantee that, from the first to the last vial filled, the material is fully identical. Therefore, a verification of the homogeneity within and between vials has to be performed. During the filling procedure, the producer should set aside vials at regular intervals. The homogeneity of the material should be tested within a single vial to ensure that successive test portions from the vial lead to similar results (within-vial homogeneity). The same study should be performed to verify that there is no difference between test portions taken from various vials (between-vial homogeneity). Besides substances of interest, the study

should include some matrix constituents or major and minor constituents (in the case of a matrix reference material). It is current practice for producers of CRMs (e.g. BCR) to test this homogeneity for different sample intakes. The (in)homogeneity may be estimated by examining the CV of replicate measurements obtained on samples from different vials to those obtained from test portions from one vial. These CV are also compared to the CV due to the analytical method. This CV is usually obtained by measuring the same extract or digest of one sample several times.

The above-mentioned homogeneity and stability considerations are implicitly related to the storage vial. Vials may be sealed glass ampoules (e.g. for materials containing organic contaminants and solutions), bottles for solids (e.g. sediments, aquatic plants and animal tissues), stainless-steel or aluminum bottles for gases or any kind of protecting vial. It is advisable to avoid light or radiation interaction by using amber glass or high-density polymers as often as possible. The storage temperature should be adequate to ensure sufficient stability. Low temperatures are often desirable, but are not always necessary, e.g. large cool rooms for stocks of CRM. Cooling sometimes can even be harmful, e.g. leading to precipitation of dissolved compounds. The storage conditions together with the delivery system should be deduced from a properly conducted stability study of the material and possibly a preliminary study of the material under various conditions and in different storage vials (especially for CRM). The transport should be done in the shortest period possible. Rapid delivery systems are unfortunately expensive and are solely used for certain particular cases. With each test sample, the user should receive a reporting form to be sent back to the organizer on which he/she indicates the status of the samples as received. Temperature indicators may be added to the samples so that too high temperatures occurring in transport are detected upon arrival of the sample.

In some circumstances, storage in deep-frozen conditions seems to be the only way to stabilize unstable compounds over a long period (e.g. phenyltin compounds; see [QUE 01]); the availability of reference materials certified for these compounds will hence result in

expensive storage needs and transport conditions, which will have a direct consequence on the price of the material. In other cases, deep-freezing storage has been shown to create adverse effects to sediment materials, e.g. results obtained on sequential extraction for trace metal determinations in sediments showed that the extractability may change at very low temperatures and hence the “speciation” may be altered when materials are stored at -20°C or below [QUE 02b]; in such a case, the recommendation is to store materials in cool conditions (e.g. $+4^{\circ}\text{C}$) but not to freeze them.

2.3.2.3. Procedures to certify and assign values

The certification of reference materials follows strict rules that are described in ISO Guide 35: “the certified value should be an accurate estimate of the true value with a reliable estimate of the uncertainty compatible with the end use requirements”. Depending on the type of property value to be certified and the type of CRM, there may be differences in the approach applied. The certification of primary calibrants such as pure compounds or calibration solutions relies on the identification, the purity and stoichiometry assessment and on gravimetric methods. Matrix CRMs cannot be certified on the basis of direct gravimetric methods, since samples have to be analyzed after a total transformation or removal of the matrix; in this case, the certification will rely on analyses within one laboratory using two or more independent methods by two or more independent analysts or analyses by interlaboratory studies using one or several different methods, possibly including definitive methods (e.g. isotope dilution mass spectrometry). In all cases, only laboratories of the highest and proven quality should be involved. The different certification approaches are described in another volume [QUE 02a] and will not be repeated here. Certification of a reference material leads to a description sheet (certificate) which should cover, in particular:

- administrative information on the producer and the materials;
- a brief description of the material with main properties and its preparation;
- the intended use of the materials;
- information for correct use and storage of the CRM;

- certified values and confidence limits;
- other non-certified values (optional);
- analytical method(s) used for certification and technical evaluation of the results;
- identification of certifying institute(s);
- legal notices and signature of the certifying body.

2.3.2.4. *Correct use of reference materials*

Reference materials might be used for different purposes in measurement quality, such as for method validation, calibration, estimation of measurement uncertainty, training, internal QC and PT. Different types of reference material are required for different functions. Method validation and measurement uncertainty make substantial use of reference materials for the estimation of bias (the difference between the measured value and the true value). Traceable certified values would be desirable for this aim, but lower-quality CRMs may also be used, provided matrix type and analyte concentration are within the scope of the method to be validated. Reference materials can be used for trouble shooting when some unexpected results are obtained for training purposes or for checking the correct use of a method.

Pure substance reference materials are generally used for calibration of the measurement stage of a method. The uncertainty associated with the reference material purity will contribute to the total uncertainty of the measurement. Reference materials give also a major contribution in QC and QA. For this purpose, homogeneity and stability are essential, while the certified property value is desirable, but often not possible. Certifying the property values of PT samples is often in fact too expensive and therefore consensus mean values are used instead, carrying some undisclosed elements of uncertainty.

As the certification of reference materials is a complex and time-consuming process, the number of materials and their availability is limited and generally smaller than their demand. Therefore, the user must carefully consider and choose the most suitable material

available, which might not completely match the original requirements. In assessing the appropriateness and fitness for purpose of any reference material based on the customer and analytical requirements, the factors to be considered are diverse: measurand including analyte, measurement range, matrix match and potential interferences, sample size, homogeneity, stability, measurement uncertainty and value assignment procedure.

Once the choice of material has been made, supporting information on the use of the reference material is reported in the (possibly ISO guide 31 compliant) certificate and in the (possibly ISO guide 35 compliant) report on characterization, certification and statistical analysis procedures. In the certificate, the user can find instructions on how to correctly handle the material (if special precautions are necessary), how to mix the sample when opening it (to assure homogeneity), how to dry the material (for the determination of the dry mass correction), how to correctly store the material (to ensure the stability within the shelflife indicated in the certificate), what the minimum sample intake of the material is as well as the correct use of the certified value and uncertainty.

The following section provides background information on available CRMs that are relevant for the QA/QC of marine monitoring analyses. The purpose is not to give full details about the materials and certified values but to highlight the types of materials that have been produced so far. Certified values and details about the materials can be found in [PEL 11].

2.3.3. CRMs for trace elements in nutrients

2.3.3.1. CRMs for trace elements in seawater

The analysis of trace elements in seawater is a challenge due to the low concentrations of analytes and the strong interference of major constituent ions in seawater. Seawater CRMs are therefore essential for underpinning high-quality measurements [PEL 11]. Nonetheless, there are few seawater CRMs available for trace metals. Estuarine water CRMs have been produced by JRC-IRMM, the Laboratory of

the Government Chemist (LGC) (UK) and NRCC (see [PEL 11]). The LGC6016 material (collected from the Severn Estuary, UK) is certified for cadmium, copper, manganese, nickel and lead and has higher concentrations than the BCR-505 and NRC-SLEW3 estuarine water CRMs. The BCR-505 material was collected in 1992 in the Tagus Estuary and is certified for cadmium, copper, nickel and zinc [QUE 96a]. SLEW-3 was sampled in San Francisco Bay at a depth of 5 m and is certified for 11 trace elements including the elements listed above and arsenic.

Coastal water CRMs are also available from JRC-IRMM and NRCC. BCR-579 is only certified for total mercury [KRA 98], whereas the CASS-5 material is a nearshore seawater certified for 10 trace elements.

There is a lack of CRMs for offshore and ocean waters [PEL 11]. The concentrations of trace elements in available CRMs are higher than generally found in the surface waters of the open seas [GRA 99, MIL 06], so these materials are not optimal for supporting trace element analysis in open ocean studies.

Analytically, estuarine waters may behave differently to offshore waters due to, for instance, higher concentrations of dissolved organic matter and complexation [KRA 94]. Consequently, in selecting the most appropriate CRM for seawater analysis, it is important to match matrix and concentrations with samples that are as practical as possible [PEL 11]. Seawater CRMs are generally filtered and acidified during preparation. It is critical to follow the producer's guidance when subsampling CRMs and this may require the use of clean facilities to avoid contamination. Furthermore, accurate CRM determinations do not necessarily infer good quality analysis, as errors that may arise in the sampling and/or sample handling process may not be accounted for. This can be critical for trace element analysis in seawater [PEL 11].

2.3.3.2. CRMs for nutrients in seawater

There are very few marine reference materials that are certified for nutrients. NRC has produced the MOOS-1, which is a seawater certified for orthophosphate, silicate, nitrite and total oxidized

nitrogen (TOxN). VKI (Eurofins Miljø A/S, Denmark) has also produced reference materials for estuarine and coastal waters, which are certified for ammonium, nitrite, TOxN, total nitrogen and orthophosphate, total phosphate and silicate (see details in [PEL 11]).

2.3.3.3. CRMs for trace elements in aquatic biota

There are number of (certified) reference materials commercially available to support analytical quality control in laboratories engaged in monitoring trace elements in marine biota [PEL 11]. Mussel tissue is a widely-used monitoring matrix for trace elements in the marine environment, and hence various mussel reference materials are available, even in a frozen state (although the frozen SRM 1974b from NIST is not available in Europe). The oyster tissue, SRM 1566b from NIST, is certified for 23 elements and methylmercury. NRC produces two lobster hepatopaneas materials, one of which is defatted. Other reference materials include one marine algae reference material (BCR-279) from JRC-IRMM and a fish otolith material (NIES-22) from NIES with reference values for cadmium, copper, lead and zinc [YOS 00].

There are a number of available fish materials, including some freshwater fish reference materials, that can be used in support of monitoring programs. These include three BCR tuna reference materials. Fish liver is often a target matrix for monitoring programs and OSPAR's JAMP Guidelines for Monitoring Contaminants in Marine Biota [OSP 99] stipulates liver as the preferred matrix for determination of target trace elements other than mercury. Nonetheless, DOLT-4 produced by NRC in Canada is the only liver tissue currently available. Laboratories should be aware that CRMs may differ from the matrix routinely analyzed due to processing of the reference material. Most materials are freeze-dried and some CRMs have also undergone acetone extraction to remove fat [PEL 11].

Most certified/reference values are for total metal concentrations and there are many materials available for the trace elements that are most commonly included in monitoring, such as total mercury, cadmium, lead, arsenic, copper, zinc and nickel. Furthermore, with techniques such as ICP-MS now widely used, a much broader suite of

elements can be measured at little extra cost. Many materials have certified and/or reference concentrations for a broad range of elements. Many elements, for example chromium, arsenic and mercury, can exist in various oxidation states and/or in compounds that can exhibit different environmental behavior and toxicological properties. Therefore, these are of interest to monitoring authorities. Cost and analytical issues often preclude their incorporation into routine monitoring and the limited availability of appropriate reference materials to underpin QC may be a barrier [PEL 11]. Most reference materials are certified for total mercury and many are also certified for methylmercury [QUE 96b]. With some notable exceptions, organic species of arsenic, such as arsenobetaine in fish and arsenosugars in marine macroalgae, typically predominate in marine biota and these forms are generally considered of low toxicological risk to consumers. Consequently, monitoring the more toxic chemical species, and in particular inorganic arsenic, is of interest to laboratories engaged in seafood testing. Most available materials are certified or provide reference concentrations for total arsenic, but only BCR-627 (tuna) (JRC-IRMM) provides certified values for other arsenic species, namely arsenobetaine and dimethylarsinic acid.

2.3.3.4. CRMs for trace elements in sediment

There are many aquatic sediment materials with certified and/or reference values for trace elements and too many to list here. A non-exhaustive overview of the available (certified) reference materials for inorganic constituents in estuarine or harbor sediments and in marine sediments is given in [PEL 11]. These materials are produced by IRMM, NIST, IAEA, NRC, NIES, LGC, National Water Research Institute (NWRI) and Chinese National Research Centre for Certified Reference Materials (NRCCRM; available through LGC), although the material produced by NIES is not available overseas. Sediment testing laboratories should be aware of the many reference materials available and choose materials suitable for their own needs.

Except for ERM-CC580 (JRC-IRMM), which is certified only for mercury and methylmercury and represents high mercury contamination levels [QUE 98], all other materials are provided with

certified, reference or indicative values for a variety of inorganic constituents (heavy metals and some other transition metals). Heavy metals are well characterized, being certified in most of the materials. Lanthanoids are certified in BCR-667 (JRC-IRMM). In this material, heavy metal concentrations are indicative. NIST produces a marine sediment reference material (SRM 2703), which is primarily intended for use in evaluating analytical methods for the direct determination of selected elements in solid samples of aquatic sediment and dissolution techniques using small sample sizes. For larger sample sizes, the equivalent SRM 2702 should be used and the latter material generally has much tighter uncertainties quoted. LGC6137 is a sediment from the Severn Estuary certified for extractable metals (metal soluble in hot Aqua Regia using methods based on ISO 11466 (1995)).

Materials produced by NRCC (HISS-1, MESS-3 and PACS-2) provide good coverage for trace elements, including certified values for heavy metals as well as other transitional, alkali and alkaline earth metals.

2.3.4. CRMs for organic non-halogenated compounds

2.3.4.1. CRMs for PAHs in biota and sediment

Available CRMs for PAHs in sediment and biota have been reviewed by De Boer and McGovern [DE 01] and Pellizzato *et al.* [PEL 11]. They typically provide certified/reference values for parent PAHs with between two and six rings. All the biota materials, with few exceptions, have some values for alkylated PAHs [PEL 11]. The most comprehensive coverage of PAHs in a biota CRM is NIST SRM 1974b. This mussel tissue is certified for 22 PAHs and reference concentrations are provided for another 16 PAHs. However, this material is frozen and not available in Europe. NIST freeze-dried mussel tissue CRMs are also available, including SRM 2977 which has lower concentrations than SRM 1974b. The IAEA produced four biota materials with recommended values for certain PAHs and information values for other PAHs, although reported 95% confidence intervals are often wide. These materials are a mussel tissue, two fish tissues and a seaweed (*Fucus* sp.). It is perhaps surprising to see the

lack of mussel tissue available given the widespread use of mussels for PAH monitoring programs globally. Furthermore, there are three materials for fish tissue, although PAH determination in fish tissue is not widely incorporated in monitoring programs due to the capacity of fish to rapidly metabolize PAHs [PEL 11].

There are a limited number of CRMs for sediment and materials formerly available from NRCC [DE 01] are no longer available. Currently-available sediment materials for PAHs are from harbors and freshwater or estuarine environments with only SRM 1941b, taken from the mouth of Baltimore Harbour, listed as a marine sediment. Thus, the materials are not ideal for use in wider marine monitoring programs where lower concentrations may be routinely measured [PEL 11]. Again the NIST materials provide the greatest coverage of PAHs, with 52 values listed for SRM 1944, of which 24 are certified. BCR-535 is a freshwater sediment certified for seven parent PAHs [WEG 99].

2.3.4.2. *CRMs for organotin compounds*

Monitoring of the antifoulant TBT by chemical means in biota and sediment generally includes the determination of its degradation products dibutyltin (DBT) and monobutyltin (MBT). The pesticide triphenyltin (TPhT) is also often incorporated in organotin analysis; it should be noted, however, that this compound is prone to instability [MOR 99, QUE 01]. CRMs for organotins in biota and sediment have been produced by JRC-IRMM, NRC and NIES, although NIES materials are not available outside Japan. There are three sediment materials available from NRC, a harbor sediment (PACS-2) with high concentrations of TBT and DBT, and coastal sediments mixed with PACS-2 (SOPH-1 and HIPA-1). CRM 462, a mussel tissue produced by JRC-IRMM and certified for TBT, DBT and MBT, is currently the only CRM available for organotins in marine biota [MOR 99]. BCR-462 and BCR-646 are sediments certified for TBT and DBT. The former is a coastal sediment and the latter a freshwater sediment from the North Sea Canal with higher TBT levels. BCR-646 is also certified for MBT and TPhT and its breakdown products [PEL 11].

2.3.5. CRMs for organic halogenated compounds

2.3.5.1. CRMs for PCBs

As for the other above-mentioned CRMs, a list of (certified) reference materials available for PCBs in biota is reported by Pellizzato *et al.* [PEL 11], in particular a total of 20 materials, five mussel tissues, one whale blubber, three fish oils, 10 fish tissues and one sea plant (*Fucus*). The materials were principally produced by NIST, IAEA, NRC, JRC-IRMM, CIL and Wellington Laboratories. The whale blubber SRM 1945 (NIST) provides the highest number of certified PCB concentrations, i.e. 36 PCBs congeners, although there are difficulties in distributing this material overseas due to trade restrictions on marine mammal products. The other NIST materials also offer a wide range of certified PCBs, as do the materials produced by CIL, although the latter quotes a much higher uncertainty. European materials certified include fish oils (BCR-349 and ERM-BB350), mussel (BCR-682) and herring (BCR-718) [VAN 06].

Ten (certified) reference materials for PCBs in sediments are reported by Pellizzato *et al.* [PEL 11]: three materials are produced by NIST, three by IAEA, one by JRC-IRMM, CIL, Wellington Laboratories and RTC. Of the 10 materials available, only SRM 1941b (NIST) is a marine sediment, all the others being freshwater, estuarine or lagoon sediments. The most comprehensive CRMs are those from NIST, with SRM 1944, SRM 1941b and SRM 1939a, certified for 25, 28 and 64 PCB congeners, respectively. The heavily-contaminated sediment EDF-5184 by CIL offers also certified values for many PCB congeners, but wide uncertainties are quoted. The materials provided by IAEA provide recommended or informative values.

2.3.5.2. CRMs for PCDDs, PCDFs and dioxin-like PCBs

Pellizzato *et al.* [PEL 11] reports (certified) reference materials for dioxins and furans for biota and sediment, respectively. With regard to biota, a comparison with the materials available in 2000 [DE 01] indicates that, with the exception of CARP-2 replacing CARP-1 and the new fish tissue WMF-01 by Wellington Laboratories, the situation

is almost unchanged. This confirms the difficulty in certifying these analytes.

The materials available are the cod liver oil SRM 1588a (NIST), carp tissue CARP-2 (NRC) and three fish tissue materials with different degrees of contamination (CIL), and the above-mentioned fish tissue WMF-01 (Wellington Laboratories). SRM 1588b by NIST has reference values for several dioxin congeners and octachlorodibenzofuran. The carp tissue has reference values only for dioxins. The three CIL materials have certified values for a range of furans and dioxin congeners, although the reported uncertainties are wide. WMF-01 has certified values only for three dibenzo-*p*-dioxins and two dibenzofurans; all others are provisional values for information only, which might be certified at a later stage. The dioxin-like PCBs are certified in the CIL and Wellington Laboratories materials and in BCR-719 (JRC-IRMM). Reference values are reported in SRM 1974b (NIST), SRM 1945 (NIST) and in the sea plant homogenate IAEA-140 OC for PCB 77, and in SRM 1945 (NIST) for PCB 126 and PCB 169.

The availability of (certified) reference materials for dioxins and furans in sediments has decreased considerably since 2000 [PEL 11]. A number of materials are no longer available, in particular those produced by NWRI. Therefore, there are currently only three materials with values for dioxin and furans. These are the SRM 1944 (NIST), EDF-5184 (CIL) and WMS-01 (Wellington Laboratories). The values are reference for the first and certified for the second and third. Dioxin-like PCBs are certified in EDF-5184 (CIL) and WMS-01 (Wellington Laboratories), and SRM-1941b (NIST) provides a reference value for PCB 77.

2.3.5.3. CRMs for BFRs

Eight biota reference materials are reported by [PEL 11]. NIST has provided certified or reference values in four materials: cod liver oil, whale blubber, fish tissue and mussel tissue; providing certified or reference values for around 15 brominated diphenyl ethers (BDE) congeners. CIL has certified certain BDE congener concentrations in their three fish tissue materials, although the reported uncertainties are

wide. Wellington Laboratories have recently made the new CRM WMF-01 commercially available. There are only two (certified) reference materials for brominated flame retardants available for sediments, and only EDF-5184 (CIL) provides certified values.

2.3.5.4. *CRMs for OCPs*

Pellizzato *et al.* [PEL 11] report 15 materials available, which represents an improvement in comparison to the only eight materials available in 2000 [DE 01]. Of those 15 materials, seven were produced by NIST (three mussel tissues, one cod liver oil, one whale blubber and two lake fish tissues), four by IAEA (fish, mussel, tuna and *Fucus* homogenates), one BCR (cod liver oil), one by NRC (carp) and two (fish tissues) by CIL. SRM 1588b and SRM 1945, and 1946 and 1947 by NIST offer the greatest coverage of certified values for OCPs. EDF-2524 and EDF-2525 also offer a very extensive coverage, but with uncertainties that are much higher than for the NIST materials. Indicative or recommended values are provided for the IAEA materials and for CARP-2 (NRC). The availability of OCP reference materials for sediment matrices has also increased in comparison to the year 2000. There are currently six materials with certified or indicative values for OCPs (see [PEL 11]). These include three NIST materials such as the marine sediment SRM 1941b, and three sediments by IAEA. The best coverage of certified values is offered by SRM-1941b (NIST). All others materials provide only non-certified values.

2.3.5.5. *CRMs for chlorinated benzenes*

Chlorinated benzenes are ubiquitous hydrophobic chlorinated organic pollutants, which can enter the aquatic environment as solvents and byproducts of phenol and pesticide manufacturing. Among these compounds, HCB, due to its persistence, bioaccumulation potential and toxicity, has been placed on various lists as a substance of concern [PEL 11]. These include listing in Annex X of the WFD as a priority hazardous substances and as one of the initial 12 persistent organic pollutants under the Stockholm convention. The analysis of this class of compounds is still quite problematic. As a consequence, the availability of CRMs for chlorinated benzenes in biota and sediments is very scarce. Only HCB

is certified in some organochlorine pesticide biota materials. IAEA provides some biota materials with recommended or information values for HCB (see [PEL 11]). With regard to sediments, HCB is certified in SRM 1944; information or recommended values for HCB are reported for three sediments produced by IAEA. NWRI materials (EC-1 to EC-8) have reference concentrations for many di- and trichlorobenzenes, pentachlorobenzene, hexachlorobenzene, hexachlorbutadiene, hexachloroethylene and octachlorostyrene, with EC-2 and EC-3 providing certification of a number of these analytes [DE 01]. Although many of these materials can still be acquired from NWRI to support laboratories undertaking these analyses, they are no longer certified due to insufficient participation in PT exercises to underpin the certification process [PEL 11].

2.3.6. Future needs of CRMs

Although progress has been made in the last decade and some needs have been fulfilled, such as new CRMs for PBDEs, there have been regulatory developments and additional emerging pollutants have been included in monitoring frameworks such as the OSPAR JAMP and WFD [PEL 11]. For instance, there are very few seawater CRMs available to laboratories required to determine WFD Annex X substances in coastal and transitional waters to assess compliance with EQS [BER 03]. Therefore, there is the necessity to produce new certified materials or certify already-existing materials for those substances. Needs related to some of the emerging class of organic compounds are reviewed by Pellizzato *et al.* [PEL 11] and will not be repeated here. It should be stressed that current gaps in reference materials represent potential weak links in the traceability chain of marine monitoring analyses. This is discussed in last chapter of this book.

Types of Monitoring

3.1. Classical chemical marine monitoring

3.1.1. Introduction

Moffat *et al.* [MOF 11] review classical approaches to marine monitoring. “Classical monitoring” is meant to refer to the usual sampling/sample treatment/laboratory analytical framework, which covers different technical features about sample collection and treatment, sample storage and laboratory analyses. This section provides a general overview of monitoring carried out in a “classical” way. Other approaches such as biomonitoring and *in situ* monitoring are described in the following sections.

Marine monitoring objectives (as described in Chapters 1 and 2) are based upon analytical measurements, some of which require the isolation of trace compounds from a complex matrix that, in itself, represents only a small component of a larger population or environment. Other requirements are related to trend studies from well-defined locations over a given number of years [MOF 11]. Different monitoring approaches are all faced with technical challenges in sampling, sample treatment, analysis and, ultimately, interpretation of data obtained. Ideally, monitoring data should be obtained in a well-structured quality assurance (QA) framework such as the one described in Chapter 2, and sufficient data should be

collected to obtain a statistically representative number for mapping or trend purposes. However, sampling in the marine environment is expensive and the area/volume that is being assessed is often large, meaning that the sufficiency of data to make for confident assessments is often far from ideal [MOF 11]. This has improved over recent years with the production of technical guidelines, QA procedures and agreements about assessment criteria, which improved status assessments of marine regions. Despite the remaining gaps, the reliability of classical chemical techniques is now considered to be appropriate regarding sample collection, treatment and storage, and analyses, and chemical data can be reasonably compared with relevant assessment criteria. In turn, conclusions can be drawn on more solid grounds for the development of strategies and an improved management of the marine environment [MOF 11].

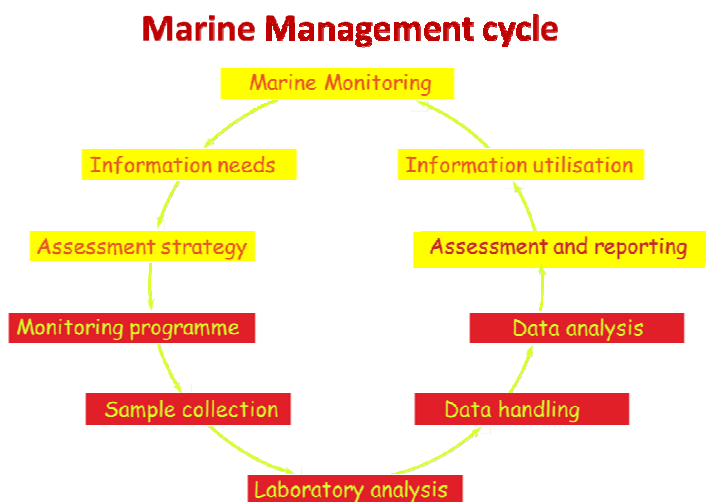


Figure 3.1. Marine monitoring steps

3.1.2. The basis and purpose of marine monitoring

As readily described in Chapters 1 and 2, marine monitoring generally aims to meet local, national and international commitments, as well as scientific purposes. As a reminder, let us reiterate the

various reasons for which chemical monitoring is carried out [MOF 11]:

- assessment of status or condition, often across a specified spatial area;
- assessment of temporal trends;
- assessment of the impact of legislation and implemented measures;
- compliance with various international agreements (see Chapter 1).

The various monitoring requirements and substances of concern related to different international conventions or the Water Framework Directive (WFD) are described in Chapters 1 and 2 and will not be repeated here. The following general monitoring definitions have been proposed [EUR 95]:

– *Monitoring*: Long-term, standardized measurement, observation, evaluation and reporting of the aquatic environment in order to define status and trends.

– *Survey*: A finite duration, intensive program to measure, evaluate and report the quality of the aquatic environment for a specific purpose.

– *Surveillance*: Continuous, specific measurement, observation and reporting for the purpose of water quality management and operational activities.

The following types of monitoring have been distinguished [EUR 95]:

– *Trend monitoring*: Measurements are made at regular, well-spaced time intervals in order to determine the long-term measurement in a particular parameter.

– *Baseline monitoring*: Used to characterize existing water quality conditions and establish a database for planning or future comparisons.

– *Implementation monitoring*: Used to assess whether activities were carried out as per the plan.

– *Effectiveness monitoring*: Used to evaluate whether the specified activities had the desired effect.

– *Project monitoring*: Assesses the impact of a particular activity or project.

– *Validation monitoring*: Deals with the quantitative evaluation of a proposed water quality model to predict a particular water quality parameter.

– *Compliance monitoring*: Used to determine whether specified waterquality criteria are being met.

Thus, different types of monitoring have to be considered according to their respective objectives, some of them embedded into regulations such as the WFD. In this context, the monitoring network is designed to provide a coherent and comprehensive overview of chemical status, i.e. EU Member States are required to monitor parameters that are indicative of the status of relevant quality elements. Within the WFD, the following three types of monitoring are mandatory:

- surveillance monitoring;
- operational monitoring;
- investigative monitoring.

In brief, the objective of the surveillance monitoring is to provide information on long-term changes in natural conditions and those resulting from widespread anthropogenic activity as well as providing information on the design of future monitoring programs. Operational monitoring is undertaken to establish the status of WFD water bodies that have been identified as being at risk of failing to meet their environmental objectives and to assess the changes in status of those water bodies as a result of programs and measures. Finally, investigative monitoring is carried out to ascertain the cause and effects of a failure when either the reason for exceedance is unknown or the magnitude of accidental pollution is unknown. Guidance on monitoring design related to surveillance, operational and

investigative monitoring is provided by the European Commission [EUR 09]; it covers a selection of monitoring points and monitoring parameters as well as detailing the procedures for the actual sampling of water, sediments and biota. Furthermore, the guidance highlights that the standards for monitoring of quality elements for physicochemical parameters will be “any relevant CEN/ISO standards or other national or international standards, which will ensure the provision of data of an equivalent scientific quality and comparability”. The methods have to be validated and, for chemical monitoring, certain minimum performance criteria have to be met. It is heartening to note that this drive for minimum standards in classical chemical monitoring is more or less uniformly recognized and where there is information already provided, this is utilized in compiling guidance [MOF 11].

In the context of the monitoring requirements of international conventions and EU regulations, it is apparent that classical chemical monitoring has, and will continue to have, a pivotal position in determining the status and trends in the global seas and oceans. That is not to say that this particular type of monitoring will not be enhanced, or, in some cases, replaced by modern technologies such as remote sensing or automated *in situ* analyses. Furthermore, there is a need to understand the impacts of hazardous substances on biota and so “biological effects monitoring” may replace classical chemical monitoring in some instances. However, the more recent technologies and effects methodologies will generally require validation through classical chemical monitoring and, in some instances, it is unlikely that classical chemical monitoring will be superseded by alternatives for many years to come. It is, however, worth briefly considering and contrasting current classical chemical monitoring with the more recent technologies and methodologies [MOF 11].

3.1.3. Some considerations around classical monitoring

Classical chemical monitoring covers a vast array of measurements using many different techniques, which have been reviewed in depth by Moffat *et al.* [MOF 11] and will not be repeated here. As stressed above, the ultimate aim of monitoring is the production of quantitative

outputs in the form of data that are used to assess status and trends with respect to water chemistry, eutrophication, hazardous substances and phycotoxins. The components investigated cover water, sediment and biota (primarily fish and shellfish but also marine mammals [PIE 08, LAW 10], seabird eggs [BEC 89, PUS 05] and seaweed [CAL 02]), while the techniques incorporate methods that allow normalization of data as well as those that produce concentrations of various compounds in different matrices. For some parameters/methods, sample preparation (extraction/isolation of the analyte and associated clean-up) is relatively simple [MOF 11]. An example is the determination of salinity, which is determined using a salinometer with no specific sample preparation [UNE81]. In contrast, other techniques, such as the determination of PAHs in shellfish, involve a complex procedure to extract the PAHs and provide a sample that can be injected onto a gas chromatographer. Different methodologies used to measure the various compounds of interest to those undertaking marine assessments are described in detail in Chapter 4.

Classical chemical monitoring is generally characterized by discrete sampling and compound-specific analysis. This results in the generation of data that do not provide access to complete information since the methodologies do not generally isolate only the bioavailable fraction nor do they provide information on the range of compounds to which an animal may have been exposed [MOF 11]. Rather, classical chemical analysis of hazardous substances provides information on the concentration of a specific compound or group of compounds. For example, there are 209 individual PCB congeners, of which only a limited number of them are monitored. Some of the limitations can be overcome through the use of new technologies such as passive sampling (see section 3.3) or by making measurements that provide some indication of the biological effects of hazardous substances (see section 3.2), e.g. the impact of the (now banned) hull antifoulant TBT is best investigated through its biological effect (the “imposex” phenomena developed by the female marine snails such as the dog whelk *Nucella lapillus*), since this biological effect occurs at concentrations that are difficult to detect by classical chemical methods. A characteristic of the marine environment is the variability

that exists both spatially and over time. Some of the variation is well documented with relatively long time scales. For example, nutrient concentrations are high in the winter months and low in the summer months, the difference of which can be measured with weekly sampling and classical chemical analysis in the laboratory but not necessarily more detailed changes in nutrient concentrations [MOF 11].

In conclusion, classical chemical monitoring, biological effects methodologies and *in situ* technologies all have their own benefits and limitations (Table 3.1). The viability of any methodology is dependent on many factors including cost, availability of the technology, delivery of data of appropriate precision and accuracy, and the availability of relevant assessment criteria [MOF 11]. Ultimately, any methodology must be “fit for purpose” and this means providing data that are interpretable such that it is possible to come to conclusions on status and trends. It is likely that future monitoring programs will be a mixture of classical chemical monitoring, *in situ* monitoring and biological effect determinations. The challenge will be integrating the different methodologies to provide truly holistic assessments.

<i>Characteristic</i>	<i>Classical chemical monitoring</i>	<i>In situ chemical monitoring¹</i>	<i>Biological effects monitoring</i>
Type of sampling	Spot sampling, often from a ship	Single site using, for example, Smart Buoy	Spot sampling, often from a ship or 30 min trawl
Frequency of sampling	Medium (weekly) or low (monthly or annual)	Very high (15 min) or high (hourly)	Low (monthly or annual)
Spatial coverage	Good	Limited by the availability of <i>in situ</i> monitoring devices	Good
Temporal resolution	Limited	Good	Limited
Components	Water, sediment, biota	Water	Biota
Analytes	Hazardous substances, nutrients, salinity, chlorophyll, biotoxins	Nutrients, salinity, chlorophyll	Indicators of exposure to hazardous substances, e.g. imposex, EROD, vitellogenin, acetylcholinesterase activity, scope for growth, etc.

Benefit	<p>Large number of analytes in water, sediment and biota</p> <p>Technical guidelines available</p> <p>Well established methodologies and quality assurance</p> <p>Assessment criteria available for many analytes</p>	High frequency of monitoring giving excellent temporal resolution	<p>Can provide information on exposure to a specific contaminant (e.g. tributyltin (TBT))</p> <p>Provides information on the impact of exposure to the environment in which the animal lives</p> <p>Takes account of what is bioavailable</p>
Limitation /problems	<p>Single compound or group of compounds</p> <p>Limited temporal resolution</p> <p>Requires a sampling platform, often a ship</p>	<p>Analytes currently limited to nutrients, salinity and chlorophyll</p> <p>Subject to biofouling that limits operation</p> <p>Limited spatial resolution</p>	<p>Many techniques still being developed</p> <p>Limited number of assessment criteria available</p> <p>Cause and effect can be unclear</p>

1 Passive sampling is not included in this summary. Although it provides *in situ* sampling, the determination of the contaminant concentration relies on classical chemical analysis.

Table 3.1. Comparison of classical chemical monitoring with “effects” monitoring and continuous monitoring processes (according to [MOF 11])

3.1.4. Designing a sampling program

An efficient protection regime of the marine environment relies on the ability to detect trends, describe water bodies, compare one sea area with another and describe any difference with respect to concentrations of persistent organic pollutants (POPs) and to be in a position to state if changes in practices and procedures have resulted in improved environmental status for marine waters [MOF 11]. This implies that representative samples (principally water, sediment and biota) are collected and analyzed. Classical chemical analysis is characterized by collecting discrete samples, which is generally costly and time consuming as it often requires a ship (which risks that the sampling process is hindered by bad weather) and specialized samplers (e.g. for collecting seabed sediment), taking into account the

fact that sampling is blind, hence not enabling the seabed being sampled from to be observed. Optimal sampling regimes have to be developed with a sound statistical basis such that the optimal number of samples is collected in a way that ultimately allows for a description of a designated area to be made and which also permits comparisons with other areas [MOF 11]. Over the past decade, understanding has improved on how to sample the marine environment using statistical tools so that appropriate sampling protocols may be designed for the production of data that can be usefully used to describe the status of the seabed and to detect trends [MOF 07].

Sampling in the same place at the same time each year is essential; the idea is to control the sources of variation which are not of interest, such as seasonal variation, and to focus on long-term trends [PHI 80]. With the introduction of monitoring of sediment contaminant concentrations, benthic communities and biological effects, the concept of monitoring a network of fixed stations annually was enlarged, with sediment networks sometimes more densely populated so that spatial trends could also be investigated. However, over time it has become clear that sampling in the same place at the same time each year gives less control on the unwanted sources of variation than had been envisaged. In particular, many time series were shown to have significant random between-year variation and changes in concentration from year to year with no particular pattern. This, often dramatically, reduced the power (or probability) of long-term trend detection [FRY 93]. Random between-year variation and its effect on power is still an issue. Detecting a doubling or halving of contaminant concentrations by annual monitoring over 10 years has often been used as a convenient target for monitoring programs [FRY 97]. However, the assessments leading up to the OSPAR QSR 2010 found that, for example, only one-third of mercury time series and one-quarter of CB153 time series in biota met this criterion [MOF 11].

It has been difficult to identify the main causes of random between-year variation, although this is fundamental to improve the power of trend monitoring programs. This may actually arise from the

“same place–same time” sampling philosophy, e.g. in sediment monitoring, if several grabs are taken each year from effectively the same spot, but that spot varies from year to year due to navigational error, then any short-scale spatial differences in concentration between the spots (e.g. due to differences in substrate, currents or deposition) will be modeled as between-year variation [MOF 11]. A similar effect arises if all samples from a station are analyzed in the same batch, but there is variation in analytical performance between batches. A simple way to reduce this effect is to take samples over a larger area, or at several times, and to analyze them in several batches. This should reduce the between-year variation and lead to designs for monitoring programs with better power. Over the past two decades, monitoring objectives have gradually shifted toward making regional assessments of both status and trends. However, current monitoring networks are often unsuited to this objective since the stations that cover only a small area can be far apart and are often close to point sources so are not “representative” at a regional scale. Regional assessments require a change in design with, in particular, networks of stations that are representative of the regions of interest [MOF 11].

3.1.5. Sample collection and immediate handling

In the context of marine monitoring and its related objectives, the selection and collection of appropriate samples (whether water, sediment or biota) is an integral part in assessing the marine environment. A proper assessment can only be achieved if relevant samples are collected and appropriately handled immediately following collection. Sample collection is only the first stage in a process that involves a number of steps that ultimately result in the production of scientific data and provision of information [MOF 11]. If this aspect of the process is not conducted appropriately, then there is no point in progressing to subsequent stages as any assessment process would be of little value. Thus, the handling, processing and storage of samples immediately following collection in the field should be strictly controlled.

As an example, hydrocarbon compounds are ubiquitous within the environment and also abound on sampling vessels. Thus, the

opportunities for contamination at any stage, including initial collection, during subsampling, from the storage container and during the transfer to the laboratory, are significant. Adventitious contamination can make the assessment of PAH concentrations in a sample impossible, thereby wasting considerable time, effort and money. This means that considerable care must be taken, especially when collecting material, to provide background or reference data. Samples should not come in contact with plastic, rubber or similar materials (such as gloves and bags), solvents, cleaning fluids or oils. Other preparative procedures are essential. These include rinsing sample containers with an appropriate organic solvent (e.g. pentane, hexane or dichloromethane) as well as rinsing any aluminum foil used in the storage of fish samples with an appropriate solvent. Furthermore, such solvents should be of an appropriate quality and should be tested to ensure their suitability. Although some of the procedures that have to be followed may appear to be unnecessary and time consuming, they are essential if sample integrity is to be maintained and assured.

3.1.6. Sample storage (short- and long-term)

The integrity of samples following collection is an essential part of the analytical process. This can only be achieved by appropriate storage, whether it is short- or long-term. In this respect, the storage temperature is critical and has to be adapted to different types of samples/parameters to be monitored (Table 3.2). For small sample sizes, snap freezing in liquid nitrogen is appropriate and cryogenic storage in dry containers is also very useful when undertaking field work. For larger sample sizes, e.g. bulk sediment or fish muscle, it is important that the sample is packaged so as to allow rapid cooling to the core of the sample. Once the sample has attained its storage temperature, every effort should be made to maintain that temperature throughout the lifetime of that sample. Repeated freezing and thawing of samples should be avoided; this means that the further processing of any samples should be planned with a life history of every sample being maintained [MOF 11].

<i>Type of sample</i>	<i>Treatment and storage</i>
Sediment	Frozen and stored at $\leq -20^{\circ}\text{C}$
Fish flesh, shellfish tissue and liver tissue for chemical analysis (e.g. PCBs, PBDEs, PAHs)	Frozen and stored at $\leq -20^{\circ}\text{C}$
Fish liver for biological effects measurement	Immediately frozen in liquid nitrogen and stored at $\leq -80^{\circ}\text{C}$
Fish flesh for sensory assessment	If tested within 72 hr, refrigerate at $4 \pm 2^{\circ}\text{C}$ If there is a delay in testing beyond 72 hr, then samples should be frozen and stored at $\leq -20^{\circ}\text{C}$ for a maximum of 3 months
Shellfish tissue for sensory assessment (excluding mussels)	If tested within 72 hr, refrigerate at $4 \pm 2^{\circ}\text{C}$ If there is a delay in testing beyond 72 hr, then samples should be frozen and stored at $\leq -20^{\circ}\text{C}$ for a maximum of 3 months
Mussels for sensory assessment	If tested within 72 hr, refrigerate at $4 \pm 2^{\circ}\text{C}$ If there is a delay in testing beyond 72 hours then samples should be cooked and stored at $\leq -20^{\circ}\text{C}$
Shellfish tissue for biological effects measurements	Immediately frozen in liquid nitrogen and stored at $\leq -80^{\circ}\text{C}$
Seawater for salinity determination	Room temperature, out of direct sun light
Seawater for nutrient analysis (nitrite, phosphate, total oxidized nitrogen, silicate and ammonia)	If tested within 10 hr of sampling, refrigerate at $4 \pm 2^{\circ}\text{C}$ If there is a delay in testing beyond 10 hr, then samples should be frozen and stored at $\leq -20^{\circ}\text{C}$ except for determination of silicate in which case the samples should be refrigerated at $4 \pm 2^{\circ}\text{C}$

Table 3.2. *Storage conditions for marine samples to be analyzed by classical methods for biological effect measurements and sensory assessment (according to [MOF 11])*

Samples must be packaged so as to maintain their integrity; in this respect, sample containers have to be adequately selected. In other words, the purpose of the analysis has to be considered when deciding how to package the sample and what material should be used, e.g.

when determining metals in sediment, a plastic container may be used while this is to be avoided for PAHs-related analyses. Similarly, the determination of silicate in seawater necessitates the use of plastic bottles rather than the standard glass bottles in which samples for the determination of salinity or nitrate concentration are packaged. Biological samples can be small and must be wrapped carefully in aluminum foil before being labeled and transferred to an appropriate container. The key here is simply to identify that there is a need to consider the packaging of samples since their loss or damage means that time has been wasted, a time series may have been broken and an assessment is not possible.

It is not unknown for a sample to lose its “label” at which point it can only be discarded. Labeling with “indelible ink pens” does not guarantee a permanent label, especially when samples are stored frozen and then defrosted or when you are working with wet samples. Under such conditions, an alternative should be considered, such as tie-on labels. Another problem is that a name or a code may make perfect sense at the time of writing, but some months later could well be unintelligible. The key to good labeling of samples and subsamples, especially when operating in the field, is to prepare them well in advance and to include the labeling within a recognized quality system. In this way, there is a structure to the numbering system and a prescribed method for recording the data that are then maintained in an appropriate data archive. Another methodology that can assist in ensuring accurate labeling and tracking of samples is through the use of bar coding. This can be incorporated into a laboratory information management system. To facilitate this, preprinted sample sheets may be used to ensure that all the required information is collected and recorded at the time of sampling. Such information should include, for example, a sample identification code, date and time when the sample was collected, depth from which the sample was collected (if pertinent) and location, etc. If care is taken, and attention to detail maintained during the early stages of sample collection, then the risks of errors occurring and samples becoming misidentified will be reduced. Furthermore, the correct recording of all relevant

details will enable a concise and fully accountable audit trail to be undertaken, including the necessity of “chain of custody” statements [MOF 11].

3.1.7. Laboratory analyses

By the time samples have reached the laboratory, they will have already undergone a certain amount of processing that may have included subsampling. However, it will often be the case that the actual sample analyzed (the test sample) is a subsample of the total material available. This allows, if required, the analysis to be repeated. As detailed earlier, for some classical chemical methods, the primary sample or a subsample is analyzed directly. This includes determination of salinity or nutrients. However, in the majority of cases, there is a need to extract the analyte of interest, be it a metal, organic contaminant, biotoxin or the pigments present in phytoplankton, from the matrix (see Chapter 4). The resulting extract is then subject to a clean-up process prior to instrumental analysis. The instrumental reading is then processed so as to provide a quantitative output. Finally, the data quality is checked. Specifics regarding the laboratory analysis of various analytes are discussed in Chapter 4. However, there are several common key features that should ensure data from classical chemical methods are both accurate and precise¹. These are discussed in the following (adapted from [MOF 11]).

3.1.7.1. QA procedures and method validation

QA aspects have been readily dealt with in Chapter 2. The section recalls some of the procedures undertaken by laboratory staff to ensure that data of the appropriate quality is obtained to meet the defined aims of the laboratory. There are two main components:

- *Quality control*: This is achieved by performing a number of procedures that maintain measurements within an acceptable level of

¹ Accuracy is the closeness of an experimental measurement or result to the true or accepted value. Precision is the random or indeterminate error associated with the result and is measured by the standard deviation (see [PRI 01]).

accuracy and precision. This includes the determination of recoveries for the analyte in question, an evaluation of the limit of detection (LoD) and limit of quantification (LoQ)², the use of laboratory reference materials (LRMs) and certified reference materials (CRMs), the participation in an external proficiency testing scheme, etc. (see Chapter 2).

– *Quality assessment*: This is delivered through procedures that provide documented evidence that quality control (QC) is achieved. This includes controls that are used to:

- ensure a method is under control;
- check for trends;
- highlight problems.

Integral to quality assessment is the use of control charts [MUL 03], which are a form of internal QC. An example of such a chart is the Shewart chart that is constructed on the basis of the repeated analysis of an LRM. This produces a mean value for the analyte with warning and action limits being set at $2 \times$ and $3 \times$ the standard deviation (Figure 3.2). A set of rules are put in place to ascertain whether or not a test point passes or fails. Failure can occur when:

- one point is outside either the upper or lower *action* limit;
- Two consecutive points are outside either the upper or lower *warning* limit;
- Seven points in a row are either *above or below* the mean.

² The limit of detection (LoD) for an analytical procedure is defined as the minimum single result which, with a stated probability, can be distinguished from a suitable blank value (see [PRI 01]). For an analytical method, the determination of the limit of detection is based on a multiplier of the sample standard deviation of a blank determination or the repeated analysis of a sample spiked with a low concentration of the analyte. In the authors' laboratory, the limit of detection is calculated using a numerical factor of 4.65. The limit of quantification is the lowest concentration of an analyte that can be determined with acceptable uncertainty. For most trace analysis, the level of quantification is taken as 10 times the sample standard deviation.

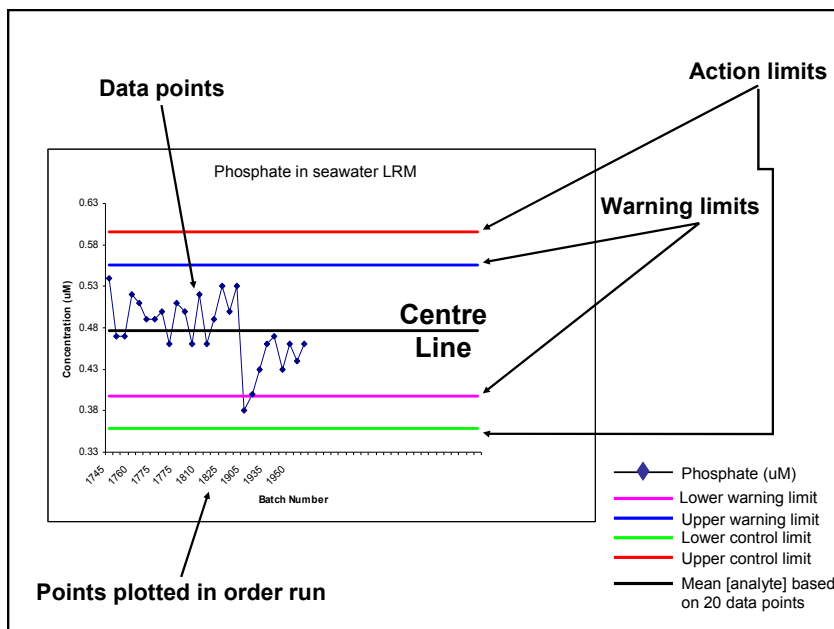


Figure 3.2. Example of a Shewhart chart used as a part of the internal quality assurance procedures when conducting classical chemical monitoring. A mean value (the center line) is determined for the specific analyte using a laboratory reference material (LRM). Warning limits and action limits are usually defined at ± 2 standard deviations and ± 3 standard deviations, respectively

Ultimately, the laboratory method being used should be fully validated. Method validation checks should be carried out on all methods before they are brought into use; in other words, they are used to analyze actual test samples. This applies to new methods, when new instruments are installed or if existing methods are significantly changed. For chemical methods, the minimum requirement for validation is the calculation of LoD, precision (reproducibility) and spiking recovery (bias). Moffat *et al.* [MOF 11] report that the method LoD is determined by analyzing at least seven reagent blanks (or low matrix samples if the blank is not detectable) extracted and run over several days, with the resulting standard deviation expressed as a concentration. The LoD is then expressed as $4.65 \times$ the blank standard deviation. LoQ values can normally be calculated using the same

information (e.g. $10 \times$ standard deviation). However, as this is an idealized value, when reporting “less than” values in test reports, it may be more appropriate for some methods to run a low calibration standard or reference material at or around the level of the theoretical LoD. This demonstrates that the idealized LoD value can be achieved on the day of analysis.

To determine reproducibility, Moffat *et al.* [MOF 11] undertake at least seven replicate determinations of a standard at the upper (90%) and lower (10%) end of the working range of the analyte(s), run “as a sample”. These are run on separate days (reproducibility conditions), and expressed as concentrations. The mean, standard deviation and percent variance is calculated and then compared with a target value.

To determine bias/recovery, the preference is to analyze at least seven replicate determinations of an appropriate CRM (see Chapter 2). If such a material is not available, then at least seven replicate determinations of a well-characterized QA sample, with a known assigned value, or at least seven replicate determinations of a sample and a spiked sample can be used. The samples are extracted and run on separate days, and expressed as concentrations. The resulting data are averaged, and the sample standard deviation calculated, and expressed as a percentage. This is then compared to a target value.

Such procedures, as outlined above, contribute to ensuring that the data used in assessments are fit for purpose. However, there is a need for other “common features” of the chemical analysis that contribute to ensuring accurate and precise data [MOF 11].

3.1.7.2. *The preparation and use of SOPs*

Standard operating procedures (SOPs) are written procedures that, put simply, describe the processes and procedures undertaken by staff in delivery of the laboratory services and cover topics such as:

- routine inspection, cleaning, maintenance, testing, calibration and standardization of instruments;
- actions to be taken in response to equipment failure;
- analytical methods;

- definition of raw data;
- data handling, storage and retrieval;
- health and safety precautions;
- receipt, identification, storage, mixing and method sampling of test and control articles;
- record keeping, reporting, storage and retrieval of data;
- coding of studies, handling of data, including the use of computerized data systems;
- operation of QA personnel in performing and reporting study audits, inspections and final study report reviews.

SOPs should be written by those undertaking the analytical procedures, but checked, and ultimately signed-off, by the laboratory manager. SOPs should not be written to explain how procedures are supposed to work, but how they work. This ensures that the information is adequate and that the document invites rather than discourages routine use. There should be a SOP on writing SOPs. This is important for consistency and efficiency. For example, it should be defined who is responsible for initiating, authoring and approving SOPs and how procedures are distributed and how the use of SOPs is enforced. Deviation from SOPs can be allowed under certain circumstances, but deviations should be approved and documented.

3.1.7.3. Looking after your equipment

To some extent, this links in with the SOPs. However, the need for calibration of laboratory balances, appropriate maintenance of pipettes and regular servicing of instrumentation has to be first identified. All equipment should be adequately inspected, cleaned and maintained. Equipment used for generation, measurement or assessment of data should also be adequately tested, calibrated and/or standardized. The frequency for calibration, revalidation and testing (performance verification) depends on the instrument itself, the recommendations from manufacturers of equipment, laboratory experience and the extent of use. For instance, a pH meter or a balance should be

calibrated before each use and the wavelength of an HPLC variable wavelength detector should be calibrated about every month or whenever the cell is removed and reinstalled. However, the whole process of ensuring the generation of appropriate data is very much intertwined with the different processes and procedures outlined.

Chemical reactions are temperature sensitive and analytes can be light sensitive. As such, an appropriate laboratory environment is critical. In this context, protection from light should be an integral part of the analysis of polybrominated diphenyl ethers; this could include the use of amber glassware and the placing of blinds over windows. Temperature regulation can be an issue, especially when operating on board a research vessel. Drift in silicate determinations at sea was found to be due to the change in air temperature in the instrument room on the ship. This was remedied by the installation of air conditioning. Staying with nutrient analysis, the use of a tungsten filament as the light source in the nutrient analyzer proved problematic due to the vibration of the ship. This was resolved by using a light emitting diode. These examples illustrate how crucial it is to look at all aspects of the analytical procedure.

3.1.7.4. *Solutions and reagents*

Many high-quality, high-purity reagents are available for purchase. However, the quality of the products should never be simply accepted; reagents should be tested and laboratory procedures established to ensure that the products being used do not introduce unwelcome contaminations or result in poor chromatography (Figure 3.3). The nature of the insert within the cap on the bottle of solvent, impurities in the sodium sulfate used to dry extracts or the cleaning product used to clean the glassware can all influence the analytical result in an adverse manner. All reagents and solutions should be labeled to indicate identity, titer or concentration, storage requirements and expiration date. The expiration date depends on the nature of the chemical. Sodium chloride has practically no expiration date. In these cases, it is generally acceptable to indicate “none” or “not applicable” on the label for expiration date. However, such a designation must be justifiable. Formal studies are not always required to justify assigned

expiration dates. It can be sufficient to assign expiration dates based on literature references and/or laboratory experience. Deteriorated or outdated reagents and solutions should be discarded. Finally, it is also good practice to include the date on which they were opened; this can be critical for some chemicals, such as ether.

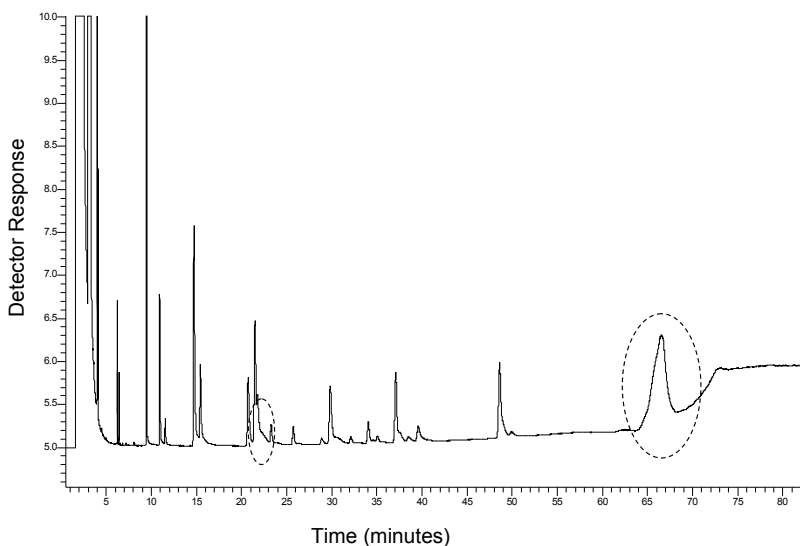


Figure 3.3. Example of a gas chromatography-flame ionization detection (GC-FID) chromatogram of fatty acid methyl esters where there is excessive peak tailing and a substantial contaminant peak at approximately 66 min. The peak tailing is often related to contamination of the retention gap, a 30 cm × 0.53 mm id silanized precolumn placed between the on-column injector and the analytical column (according to [MOF 11])

3.1.7.5. Effective document control

Integral to any quality system should be effective documentation. This can include the use of controlled laboratory notebooks as well as SOPs. All controlled documents (SOPs and worksheets) should include, as a minimum, the title, version number or date, owner and

pagination. Where appropriate, standard forms should be prepared. Worksheets should be used for recording information on the analytical batch such as the date of extraction, the weight of sample extracted and the amount of internal or recovery standard added. Worksheets can also be used for calculating the final concentration in the sample. These can be set up to automatically blank correct and adjust for the weight of the sample used. In addition, sheets can be protected so that staff are only able to insert the relevant raw data required and cannot accidentally change formulas used in the calculations. All completed records should be uniquely identified. This is especially useful when operating within a quality system when this can be linked with the issuing of sample numbers.

3.1.8. The final assessment

Determining the concentration of PCBs in fish liver, nitrate in seawater or cadmium in sediment is fundamental to the processes of assessing the status of our seas. However, there is a need to understand the relevance of the concentration that has been determined. Does the measured concentration raise concerns in terms of human health, environmental impact or the risk of eutrophication? Ultimately, the concentration has to be compared to some criteria so that a conclusion can be drawn about the significance of the data in terms of risk to the plants and animals that live in our seas. The gas chromatography with electron capture detection (GC-ECD) analysis of a sample of fish liver provides an image from which the various peaks can clearly be identified and quantified. However, the significance of the determined concentrations can only be fully understood by comparison with assessment criteria. This is described in details by Moffat *et al.* [MOF 11], in particular criteria developed by Working Groups and Committees of the OSPAR Commission (see Chapter 1) based on the derivation of background or reference concentrations (comparing concentrations with background concentrations of pristine sites, see [WEB 09]), by the ICES Marine Chemistry Working Group [ICE08], by HELCOM using contamination ratios calculated by dividing individual contaminant

concentrations (or biological effects measurements), by the relevant assessment criteria, etc. The above-mentioned criteria are only examples of the assessment criteria that are available to assess status and trends in the marine environment and have recently been used in status reports. The key point is that development of such criteria must continue and such development should be cooperative across Regional Sea Conventions so that the best available techniques are shared and, where possible, common assessment tools are used across the international frameworks that have developed to manage our global marine environment.

3.1.9. Conclusions

In conclusion, it is apparent that classical chemical monitoring will continue as an integral part of the assessment of our marine ecosystems well into the future. However, there is a need to develop relevant assessment criteria and it will require the integration of biological effects measurements with data from classical chemical monitoring. Furthermore, regardless of whether it is classical chemical monitoring, biological effects monitoring or else the use of *in situ* monitoring, there are common requirements around ensuring that all processes are appropriately quality controlled. Some of the challenges are different with respect to the different methodologies; however, without coming together of the various monitoring processes and methodologies, the advice that scientists can provide to those developing marine policy will be inappropriately limited. This requires that we all think outside the box, learn from each other and are prepared to grasp opportunities afforded by the new technologies while maintaining the high-quality classical monitoring tools where they are the best solution.

3.2. *In situ* methods

3.2.1. Introduction

Marine monitoring presents a number of challenges that are not encountered in fresh water monitoring, including spatial coverage,

complex current systems that shift with time, large dilutions of contaminants at low but often biologically significant concentrations, tidal effects in coastal zones, etc. It is recognized that the use of sampling coupled with classical chemical or biological analysis is expensive, time consuming and often fails to provide robust, representative information over changes in time (short-term over a tidal cycle, and longer term over seasons, years and decades), and sufficiently detailed spatial information over the large areas involved [GRE 11]. A further problem is that prolonged delays between sampling and analysis can compromise sample integrity, especially for labile analytes. This can be reduced where ship-board analysis is available. However, samples taken at significant depths can be modified during transport to the surface, since this can take several hours and be associated with large changes in temperature and pressure, which has driven the development of *in situ* methods that offer advantages over the classical methods [VUI 09], in particular remote *in situ* technologies that can provide continuous or semi-continuous observations, and transmit the information telemetrically to shore-based data gathering establishments. In contrast with methods that depend on collection of samples for analysis in laboratories (landbased or shipboard), *in situ* methods measure variables directly in the environmental medium. This approach has the advantage that it eliminates the need for costly (>€18,000/day) sampling from ships, and transport processes, and there can be real-time acquisition of information that decision makers can respond to problems in a short time scale. No matter what methods are applied, it is essential that appropriate QC and QA procedures are in place in order for the information to be useful for end users, including regulators [GRE 11].

The first true *in situ* analyzer was that for determining salinity on the basis of measurements of conductivity and temperature, which is considered to represent a turning point in marine chemistry [WAL 74, GRE 11]. Many *in situ* techniques are now available and are currently used for monitoring a wide range of hydrological and physicochemical variables in the marine environment, e.g. smart

buoys deployed at key sites can house a range of measuring instruments and transmit data from them telemetrically. This type of network provides long-term data that can be used for detecting trends. Variables routinely monitored include pH, pO₂, pCO₂, turbidity, conductivity and concentrations of nutrients such as phosphate and nitrate. Sensors, like any technology deployed in nutrient-rich environments (such as most coastal waters), suffer from the problem of biofouling, and this can be a limiting factor [GRE 11]. There are fewer sensors routinely in use for monitoring concentrations of heavy metals, POPs and algal toxins. In vulnerable areas, such as aquacultural installations where protection against contamination by pollutants or algal toxins is necessary, it is possible to use living organisms to provide warnings in real time.

This section aims to provide an outline of current developments in the area of *in situ* monitoring of the chemical quality of marine waters. It is based upon reviews by Greenwood *et al.* [GRE 11] and Mills *et al.* [MIL 11] available in water quality measurement [QUE 11b].

3.2.2. *In situ* automatic analyzers

A range of approaches has been developed to achieve *in situ* measurements that can be used at sites such as a river bank or a boat, e.g. miniaturization of existing analytical methods (such as colorimetric and spectroscopic methods), production of miniaturized portable instruments, miniaturized systems based on flow injection analysis (FIA) methods with capacities to be deployed *in situ*, etc. [GRE 11].

Analyzers based on FIA methods for *in situ* use comprise a pump, detector and narrow bore tube manifold [VUI 09]. The sample is pumped through a tube manifold where it is mixed with appropriate reagents to form a product that can be detected by spectroscopic methods. In some cases, the reaction product is colored and can be detected by visible wavelength spectroscopy; in others it is detected

by fluorimetry. A system of valves allows samples to be replaced by standard solutions from time to time to achieve an *in situ* calibration of the instrument and provide QC information. Some problems were encountered during the development of FIA analyzers for use *in situ* in the marine environment and various optimization developments were necessary, as discussed by Daniel *et al.* [DAN 95]. Adaptation of existing colorimetric methods for *in situ* use in seawater involved a large amount of development work to optimize the design of the manifold and optical systems. In addition, works have been needed to provide a housing to protect analyzers during deployment.

Several miniaturized analyzers have been developed for *in situ* measurement of variables, including silicic acid (silicate), nitrate and nitrite, iron(II), total iron and total sulfide, and have been used successfully in the marine environment for yielding information on the chemical quality of water in relation to the distribution of biota in the deep ocean. Examples are given below, details of which (in particular drawbacks and method performance) are provided by Greenwood *et al.* [GRE 11] and the respective method developers:

- a colorimetric method using the production of β -silicomolybdic acid that is reduced (by tin(II)) into an intensely colored molybdenum blue for the measurement of silicic acid (silicate) in seawater [FLO 98];

- an FIA analyzer for measuring ammonium in fresh and saline waters [AMI 01] based on a fluorometric method (using an ortho-phthaldialdehyde sulfite reagent);

- a system with three analyzers developed by Thouron *et al.* [THO 03] for measuring three key nutrients: nitrate, phosphate and silicate, using assay chemistries (reactions of analytes, e.g. reduction of nitrate to nitrite, conversion of nitrite to an azo dye and formation of yellow β -silicomolybdic acid that is reduced using ascorbic acid in molybdenum blue), which allows the use of micro flow rates that require only small volumes of standards and reagents compared with FIA;

– an *in situ* analyzer developed by Okamura *et al.* [OKA 01] for measuring manganese in hydrothermal plumes with a through flow system using chemiluminescence (CL) and based on manganese(II)-catalyzed luminol CL after removal of potentially interfering metals (mainly copper, iron and zinc) using an inline 7-dodecenyl-8-quinolol resin column;

– an FIA analyzer called ALCHIMIST developed by Ifremer (France) for measuring nitrate plus nitrite and total sulfide in deep marine locations near hydrothermal vents [LE 00]. The system is based on reduction of nitrate to nitrite using a cadmium–copper column and the reaction of the nitrite to form a colored azodye. Total sulfide ($\text{H}_2\text{S} + \text{HS}^- + \text{S}^{2-}$) analysis was based on the reaction of sulfides with dimethyl-*p*-phenylene diamine to form methylene blue. A dual wavelength system was used for both analytes.

Greenwood *et al.* [GRE 11] also report an *in situ* analyzer (the CHEMINI system), developed by Vuillemin *et al.* [VUI 09], which has shown promise for measuring analytes at great depths and in difficult environments. This has built on the experience gained with the systems described earlier using a photodiode detector and low power consumption circuit board in a tank at atmospheric pressure. Prototypes of this CHEMINI system have been deployed in deep sea hydrothermal environments for the measurement of concentrations of dissolved iron and total sulfide. The CHEMINI system was also used to investigate the chemical environment in mussel beds (*Bathymodiolus azoricus*) in the mixing zone adjacent to hydrothermal vents.

The combination of miniaturization, the availability of protective deployment devices and of stable, long-life power supplies, and advances in communications capabilities have enabled these analyzer systems to be buoy-based, located on robotic vehicles or towed by ships. However, the use of reagents sets limits on the time that instruments can be deployed, and adds significantly to the bulk and weight of the analytical system to be transported and deployed; there has been a move to develop reagentless methods where possible [LAC 08].

3.2.3. Passive sampling technologies

Active and passive sampling methods can be used for monitoring concentrations of pollutants in the aquatic environment. The former involve the use of low volume (50 mL to 2 L) spot (grab or bottle) sampling, and in some cases pumps to obtain large volumes (2–100 L) of water that can be passed through an extraction device to concentrate the analytes of interest. Passive methods include the use of passive samplers and living organisms (usually sedentary species such as bivalve molluscs or caged mobile animals such as fish). This section provides a short description of properties of the currently available passive samplers, and their potential for use in monitoring the chemical quality of marine waters as developed by Mills *et al.* [MIL 11]. Over the past 20 years, several designs of passive samplers have been developed to monitor a range of pollutant types (non-polar organic, polar organic, organometals and metals) present in the aquatic environment. Depending on the sampler design and time of deployment in the field, the mass of a pollutant accumulated by a device can reflect either the equilibrium concentration or the time weighted average (TWA) concentration of the pollutant in the water column. These samplers can be used to accumulate contaminants over extended deployment periods (days to months). This means that large volumes of water can be cleared by the devices, and hence the samplers effectively preconcentrate pollutants, lowering the effective level of detection of environmental chemicals. This feature could be particularly important for compounds that are sparingly soluble in water and also in the marine environment where large dilution factors can reduce concentrations to ultratrace levels. Uptake by passive samplers is driven by the freely dissolved concentrations of chemicals, and so this technology measures only that fraction. This contrasts with the use of water samples whereby, depending on the pretreatment procedures (e.g. ultrafiltration, filtration (0.45 μm) and centrifugation), different fractions can be measured. Currently, environmental quality standards (EQS) are framed in terms of total or filtered samples for different classes of pollutants. If passive samplers were to be used in a regulatory context, the EQS would have to be redefined to reflect the fraction measured by these devices. This

concentration has been often described as the biologically relevant, or available, fraction. Since the 1970s, similar devices have been used extensively for monitoring air quality and for measuring workplace exposure to toxic chemicals. Several reviews [VRA 05a, KOT 07, EST 08] and two books [GRE 07a, HUC 06] have been published on the state of the art of different passive sampling methods for monitoring both organic and inorganic pollutants in water and their field applications. In the following, an overview of the types of devices currently available is provided and their potential for monitoring different classes of pollutants in the marine environment is described (according to [MIL 11]).

Passive samplers consist of a receiving phase with a high affinity for the pollutant of interest that is exposed to the aquatic medium for a defined period of time. In some cases, the receiving phase is separated from the bulk water phase by a diffusion limiting membrane. This can help decrease the rate of diffusion and in some environments limit the degree of biofouling of the surface of the receiving phase. Three broad classes of sampler can be distinguished; those for measuring non-polar organics, polar organics and metals [MIL 11]:

- Non-polar samplers consist of an amorphous polymer for which non-polar analytes have a high affinity. Semi-permeable membrane devices (SPMDs) were the first non-polar samplers to be used for environmental monitoring on a worldwide scale [HUC 90]. These samplers consist of low-density polyethylene (LDPE) lay-flat tubing that is filled with a small quantity of the triglyceride triolein as the receiving phase. SPMDs have a large sampling area and can be used to monitor trace levels of pollutants with a log K_{ow} in a range of 3.5–8. The extracts from the devices need a clean-up step (e.g. using gel-permeation chromatography or silica column chromatography) to remove any residual triolein prior to instrumental analysis [MIL 11]. Polymer films and sheets have been used as non-polar samplers; examples include LDPE [ADA 07], silicone rubber [LES 02] and poly(oxyethylene) [JON 01]. These single-phase partition type samplers have high uptake rates, similar to those of the SPMD, for many non-polar priority pollutants. For example, 2 g of LDPE can

effectively sample, depending on the physicochemical properties of the analyte of concern, between 20 and 2,000 L of water over a deployment period. Other samplers for hydrophobic compounds include the non-polar version of the Chemcatcher (consisting of an *n*-octanol-soaked C₁₈ 3M Empore™ disk overlain by a thin LDPE membrane) [VRA 05b] and various configurations of the membrane-enclosed sorptive coating sampler [PAS 07]. Since the introduction of the SPMD some 20 years ago, there have been in excess of 250 papers describing its use in various field trials [HUC 06]. Many of the applications have recently been reviewed by Esteve-Turrillas [EST 08]. Most of the work has concerned deployments in surface waters. More recently, there have been applications of SPMDs and polymeric samplers in the marine environment. Mills *et al.* [MIL 11] give examples of the use of these devices and other samplers in measuring TWA concentrations of non-polar anthropogenic pollutants in a marine context, e.g. PAHs in waters [HAR 09, KOM 09], ultratrace concentrations of polychlorinated dibenzo-*p*-dioxins, polychlorinated dibenzofurans, and PCBs in the water column [ROA 09]. SPMDs were originally designed as monitoring tools to mimic the uptake on hydrophobic chemicals by biota. Several studies have compared the uptake of PAHs, PCBs and PBDEs by SPMDs with that by blue (*Mytilus edulis*) or green (*Perna viridis*) mussels [RIC 01, EST 08]. Most workers found that mussels are more efficient at sequestering PAHs, PCBs and organochlorine pesticides from the water column than SPMDs [BOE 05, SME 07]. Chemicals concentrated in the receiving phase of SPMDs can also be combined with a variety of short-term bioassay procedures to assess the biological effects of the aqueous contaminants. The identification of the most suitable bioassay technique (e.g. Microtox, Mutatox and *Daphnia pulex* immobilization assay) to use is an important factor for toxicity screening purposes. Følsvik *et al.* [FØL 02] reported the use of SPMDs for monitoring the soluble, bioavailable, fraction of organotin compounds.

– The second group of samplers targets polar analytes. These samplers consist of an adsorption phase overlain by a thin polar diffusion limiting membrane. Examples are the polar organic chemical integrative sampler (POCIS) [ALV 04] and the polar version of the

Chemcatcher [KIN 00, GRE 07b]. Receiving phases that have been used include particulate Oasis HLB solid-phase extraction (SPE) sorbent and activated carbon for the POCIS, and C₁₈, styrene divinylbenzene or ion-exchange resins bound into 47 mm 3M Empore™ disks for the polar Chemcatcher. A polyethersulfone microporous diffusion limiting membrane is used to cover the receiving phase in both devices. These samplers have smaller sampling areas in comparison with the SPMD and the commonly used polymeric devices. Naked Empore™ disk (with no diffusion membrane) samplers have also been used with varying degrees of success [GUN 08, SHA 09a]. Chemcatcher samplers without membranes have been calibrated for monitoring pharmaceuticals in a range of flow regimes in artificial channel systems [VER 08], monitoring of diuron, atrazine and simazine [STE 09], and polar and semi-polar pesticides [SCH 08]. Most applications of polar samplers have been in freshwater and wastewater systems, though some studies have used them in marinas, harbors, estuaries and inshore reefs. To date, most information is available for the POCIS device. However, one of the first marine applications of polar samplers was that of Kingston *et al.* [KIN 00] that investigated the concentrations of two herbicides (irgarol and diuron) used in antifouling paints in marinas. POCIS was also used to measure concentrations of industrial pollutants, pesticides and pharmaceuticals in estuarine waters [TOG 07]. An application of POCIS to measure endocrine disrupting compounds (alkyl phenols and ethoxylates, and steroids) in coastal regions of rivers, marine harbors and offshore (1.5 km) waters found integrative sampling of these compounds for up to 28 days [ARD 08]. The POCIS sampler was calibrated in a batch system in seawater and was used to monitor a wide range of veterinary medicines (including antibiotics, pesticides and biocides) over a period of a year in the region of a fish farm in the Mediterranean Sea [BUE 09]. The impact of offshore discharges of produced water from oil platforms in the North Sea was assessed using the POCIS sampler to monitor polar analytes (alkyl phenols) [HAR 09]. Although the usefulness of both the Chemcatcher and POCIS in marine monitoring applications have been demonstrated, the low uptake rates and the low concentrations that are observed in most offshore marine waters may result in

detection problems that hamper the identification of temporal and spatial trends. Their specific value will be in monitoring concentrations of polar pollutants in estuarine waters where concentrations fluctuate over a tidal cycle, making the use of spot sampling unreliable [MIL 11]. A variant of the Chemcatcher sampler has been developed and calibrated for the TWA concentration measurement of organotin compounds [AGU 08].

– The third sampler group targets freely dissolved inorganic ions (e.g. trace metals, phosphate and sulfide). In the diffusive gradients in thin films (DGT) sampler, a sorption phase is covered by a polyacrylamide hydrogel (thickness ~ 1 mm) and a $0.45\ \mu\text{m}$ filter membrane. Sorption phases include cation exchange resins for trace metals, iron oxides and titanium dioxide for phosphate and silver iodide for sulfide [ZHA 95, ZHA 98, TEA 99]. The metals version of the Chemcatcher employs a 47 mm 3M Empore™ chelating disk that is covered by a $0.45\ \mu\text{m}$ cellulose acetate filter [PER 01]. Over 200 papers have been published on the application of DGT for a wide range of inorganic analytes in sediments, soils and natural waters. The DGT technique is based on a simple device that accumulates solutes on a binding agent after passage through a hydrogel that acts as a well-defined diffusion layer [ZHA 95]. DGT devices have been used to assess concentrations of trace metals in marine waters [LAR 06, DUN 07, GON 09]; they have also been combined with oysters [OLI 02] and mussels [SCH 08] as biomonitors. The two methods provided complementary data because DGT accumulated only the dissolved species, whereas the oysters and mussels, being filter feeders, accumulated both dissolved species and metals bound to particulate material. Comparisons between the Chemcatcher and DGT were undertaken in a 28-day trial during which fluctuating concentrations of metals (Cd, Cu, Ni, Pb and Zn) were observed in frequent spot samples. This study assessed the potential of these two devices for the regulatory monitoring of trace metals in surface water [ALL 08]. It demonstrated that the two sampling devices provided similar information and were able to integrate concentrations reliably even where there were fluctuations in concentration during the deployment period.

– Special passive samplers have been developed for the detection and monitoring of algal toxins in the marine environment. This work was in response to a need of commercial producers of shellfish for human consumption. Many countries have legislation in place that requires monitoring of contamination levels in order to protect human health. The method most commonly used is the analysis of the concentrations of algal toxins in shellfish tissues. However, this is a difficult and expensive procedure, and since some toxins are metabolized by shellfish, the analysis is made more complex. An alternative is the analysis of phytoplanktonic samples. In the early 2000s, a passive sampler, the solid-phase absorption toxin tracker (SPATT), was developed to provide information on relative accumulation of toxins by biota before, during and after algal blooms [MAC 04]. These samplers comprise a polyester mesh bag containing activated polystyrene divinylbenzene resin. Other receiving phases (e.g. methacrylic ester copolymer resins for the more hydrophilic toxins) can be used to match the toxins to be monitored. These samplers have been used quantitatively to provide estimates of concentrations of a range of marine lipophilic toxins including azaspiracids, okadaic acids, pectenotoxins, yessotoxins and spirolides in mussels [RUN 09]. The extraction and analysis of the toxins are much simpler than for biota, and clean samples with few interfering compounds are provided. Applications of the SPATT sampler have been reviewed [MAC 10], and have been extended to monitoring of cyanotoxins produced by planktonic and benthic freshwater cyanobacteria, and that can potentially contaminate potable waters. The accumulation rates of toxins by the SPATT samplers have been well correlated with marine mussels, and uptake was linear (microgram per SPATT bag, or per gram receiving phase per day) over 7 days [FUX 08, RUN 09].

All samplers operate in a similar fashion. Target analytes diffuse from the bulk water phase to the receiving phase when the chemical activity (i.e. the effective concentration) of the analytes in the water is higher than their activity in the sampler. Far away from the sampler, all transport is by turbulent mixing. At short distances from the

sampler–water interface, a transition zone (the water boundary layer, WBL) exists where turbulence gradually decreases to zero, and where transport is increasingly governed by molecular diffusion. Molecular diffusion also dominates transport within the membrane and within the receiving phase. In some cases, a layer of biofouling (consisting of periphyton or even macrofauna) develops on the surface of the sampler where analyte transport is also by molecular diffusion. Water ventilation by organisms trapped in the biofilm layer may alter the hydrodynamic structure of the WBL. Further details on the various transport steps involving different available designs of passive sampler can be found elsewhere [HUC 06, NAM 05, BOO 07, WAR 07]. Principles related to exposure, kinetics, concentration calculation, etc., for various passive samplers are described in detail by Mills *et al.* [MIL 11].

Regarding validation, currently few laboratories are involved in the calibration of passive samplers, and because it is very labor intensive, there is a shortage of validated calibration data for many types of passive samplers. Considerably more calibration data are available for passive samplers for non-polar organic compounds than for those for polar organic compounds and inorganics [MIL 11]. If passive sampling is accepted for use in a regulatory context, then there is a need for the development of validation methods and an associated accreditation scheme for both those involved in calibration measurements, and those using passive samplers in the field. This would need to be organized in a way similar to that in place for analytical chemistry techniques. However, there are some obstacles to be overcome. For laboratory calibrations, there is a need for large volumes of calibration water that could not feasibly be distributed in the same way as reference materials used in the validation of analytical methods. For field trials, it may be possible to use reference sites that are well characterized and consistent in composition. When such sites have been established and characterized, it may be possible to use them for the calibration and validation of other monitoring technologies (e.g. sensors and other *in situ* methods). Interlaboratory field trials for a range of passive sampling technologies have been

undertaken at European riverine sites [MIL 11] and a passive sampler intercalibration trial has been organized by the Network of Reference Laboratories for Monitoring of Emerging Environmental Pollutants (NORMAN project: <http://www.norman-network.net>) focusing on emerging pollutants. Finally, a BSI publicly available specification [BSI 06] is available to provide guidance for end users on the field deployment of passive samplers. This has led to the consideration of the development of an ISO/CEN standard to provide guidance of the field application of passive samplers.

3.2.4. Spectroscopic methods

A number of spectroscopic- and spectrometric-based *in situ* devices have been developed for use in marine applications for measuring dissolved gases, nutrients and trace metals [GRE 11]. These are usually miniaturized versions of conventional laboratory analytical equipment but that have been fitted with robust protective housing to be able to enable the systems to be deployed in harsh environments and to withstand high pressures. A range of optical detectors can be used, which include absorbance, reflectance, luminescence, fluorescence, refractive index and light scattering. Underwater mass spectrometric detectors can also be employed, usually with a membrane inlet sample introduction system. Many of these *in situ* sensors have been reviewed in two books [BUF 00, VAR 00] and papers by Prien [PRI 07] and Moore *et al.* [MOO 09]. In the following, a brief discussion of some of the different optically and mass-based technologies available and their specific application to the marine environment is provided:

- One of the simplest optical sensing systems measures changes in refractive index. In the marine monitoring context, such detectors can be used to measure bulk particulate loads (e.g. amounts of organic and inorganic particles in the water column). These devices work on the backscattering of light principle and can be useful in estimating phytoplankton loads and degree of sediment resuspension [GRE 11]. Probably the most widely used method is optical absorption

spectroscopy. This method is often used analytically in conjunction with flow injection analyzers or auto-analyzers as described in the previous section. Absorption spectra (both visible and ultraviolet regions) in seawater are more complex than in pure water due to the influence of salts. The absorption peaks are broader and this can cause overlap of spectra between different compounds making identification harder. *In situ* ultraviolet/visible spectrometers are frequently used to monitor surface, waste and drinking water treatment processes, as well as, for nitrate, sulfide, bromine in seawater [JOH 06] and nitrate, chemical oxygen demand and total suspended solids (turbidity) directly in the water column [VAN 06].

– *In situ* fluorescence spectroscopy is also used in marine monitoring. Fluorescence spectroscopy is very sensitive but has the drawback of not being selective in the choice of excitation and emission wavelengths to be used. The broad character of fluorescence peaks usually prohibits finding an explicit excitation/emission wavelength combination that is unique to the analyte being measured. Due to their chemical structure, PAHs are inherently fluorescent (most can be excited in the ultraviolet range 240–300 nm and emit light in the range 310–400 nm range). A submersible ultraviolet fluorimeter has been designed for the measurement of PAHs in water (EnviroFlu-HC by TriOS Optical Sensors, Rastede (Germany)) and has been successfully tested for monitoring PAHs in polluted coastal waters [TED 10] but was not sensitive enough to measure PAHs (dissolved and bound to particles) in unpolluted coastal waters.

– Optodes sometimes referred to as optrodes can also work using fluorescence spectroscopy principles. Here, indicator dyes (e.g. ruthenium or platinum complexes) are employed and these are embedded or immobilized into sol-gel matrices of thin polymer layers [GRE 11]. The presence of a test analyte in the light path modifies the properties of the indicator (e.g. emission of fluorescent light) and this forms the basis of operation these sensors. These devices are frequently used to measure oxygen [PRI 07] but can also be used for pH and pCO₂ [SOT 10, SCH 07, ZHU 05].

– Infrared devices can also be used [MIZ 03]. The application of these devices is mainly for the measurement of volatile chlorinated

and aromatic hydrocarbons, and this relies on mid-infrared range optical systems. This wavelength region is particularly attractive for optical sensing, since molecule-specific information is provided by stimulation of the ground vibrational modes of organic substances. The use of Fourier transform methods enables simultaneous measurements of individual chemicals in multicomponent mixtures at concentrations in the $\mu\text{g L}^{-1}$ range. Interferences to the signal from changes in salinity and water turbidity also need to be taken into account. An *in situ* miniaturized modular Fourier transform infrared (FT-IR) device with a fiber optic sensor head has been developed to measure various volatile chlorinated and aromatic hydrocarbons in seawater. The effects of other interfering pollutants (e.g. aliphatic hydrocarbons and phenols) did not significantly affect the operation of the sensor. In addition, the long-term stability and resistance to fouling of the device were good [KRA 03].

– *In situ* monitoring devices based on Raman spectroscopy have also been developed [GRE 11]. Here, a laser light in the visible region is shone on a sample, the light is scattered and a small fraction (about 1 in 10^7 molecules) of this is shifted in frequency (the Raman shift) as the atoms in the material vibrate. The analysis of the frequency shifts (spectrum) of light reveals the characteristic vibrational frequencies of the atoms, and thus the chemical composition and structure of the sample being analyzed. As a result, Raman spectroscopy is highly specific and spectra can be recorded rapidly (within a few seconds). Raman active peaks are strongest for compounds that are symmetrical in structure and that do not have a dipole moment. In the marine environment, Raman spectroscopy has many potential applications but there are difficulties to be overcome. The Raman signal can often be weak and many chemical species present in seawater are not Raman active or are present at only trace concentrations. The technique has been used for geochemical studies in deep ocean waters [BRE 04], in particular to study hydrothermal vents and their associated bacterial mats because of the Raman response of vent gases (methane and hydrogen sulfide). Another Raman-based device has also been developed for the *in situ* analysis of organic compounds such as PAHs over a concentration range of $\mu\text{g L}^{-1}$ to ng L^{-1} [SCH 04].

– Laser-induced breakdown spectroscopy is an emerging technology with potential *in situ* monitoring applications. This uses a laser pulse to create a microplasma in the sample, then a spectrophotometer captures the transient light to identify and quantify the analytes. It is a relatively low cost method and can be easily adapted for use with automatic or robotic systems. Michel *et al.* [MIC 07] demonstrated the potential of this approach in the laboratory for the detection of calcium, potassium, lithium, manganese and sodium in seawater.

– Membrane inlet mass spectrometers have been developed for the *in situ* measurements of dissolved gases and volatile organic compounds over a wide range of ocean depths. Devices can be deployed on wide variety of platforms in different environments (fresh water, coastal and deep oceans). As they use no reagents for their operation, the spectrometers can be deployed for extended periods. Using mass spectrometers underwater is very challenging since the devices operate under high vacuum. Analytes must be transported from the pressurized aqueous environment into a vacuum system and this can place severe demands on pumps, particularly for long-term deployments of the apparatus. It has also been necessary to give consideration to the power requirements of the equipment. Two designs of mass spectrometer are used (linear quadrupole or ion trap), both with electron impact ionization [SHO 01]. A compact *in situ* mass spectrometric system (NEREUS) enables an efficient, rapid evaluation of underwater spectra [CAM 04]. Mass spectrometers can be used for the simultaneous detection of multiple species. Most applications of underwater mass spectrometry (MS) systems have been the *in situ* detection and quantification of dissolved gases (e.g. CO₂, O₂ and methane) and small volatile organic compounds (e.g. dimethylsulfide and toluene) at a range of depths in the water column [CAM 04, SHO 06, SCH 08]. Such systems offer much potential for the future in monitoring the distribution of natural and anthropogenic chemicals. However, in addition to improvements in the mass spectrometer itself, further developments, such as self-calibration of the analytical system, lower limits of detection for other traces gases (e.g. H₂ and N₂O), improved inlet system materials

(e.g. potential use of carbon nanotube membranes) and miniaturization of gas or liquid chromatographic columns that can be easily interfaced to the spectrometer system, are needed. The reduction in size of the spectrometer brings added benefits of a reduced vacuum system and associated power demands. The ability to measure low abundance isotopes (e.g. ^{13}C , ^{14}N , ^{18}O and ^{34}S) of natural elements using *in situ* mass spectrometric techniques would be beneficial in improving the understanding of the global oceanic cycling of many compounds. Higher resolution and more sensitive detectors are needed for this purpose, but a number of projects in these areas are currently on-going [KIB 04].

3.2.5. Electrochemical techniques

Electrochemical-based instruments are among the most widely used devices for *in situ* chemical analysis [DEN 09, MOO 09], and include conductometric, potentiometric and amperometric/voltammetric electrode systems [TAI 00]. Conductometric electrodes are used to measure salinity and are incorporated into a variety of commercially available Conductivity, Temperature and Depth (CTD) designs. Examples of potentiometric devices include those to measure pH [CAI 93a], sulfide (e.g. the Ag_2S electrode [REV 83]) and pCO_2 [CAI 93a]. Examples of amperometric devices include those for measuring concentrations of O_2 and N_2O [REV 88]. Electrodes to detect (free) trace metal concentrations so far lack the sensitivity required for use in the marine environment. Furthermore, the analyses are usually affected by changes in salinity.

In potentiometric devices, potential is measured with a near-zero current passing through an electrochemical cell consisting of a working electrode and a reference electrode. The potential is proportional to the concentration of the analyte. In amperometric devices (based on voltammetry), the current is measured at a fixed potential between the working and reference electrodes. The current is proportional to the concentration of the analyte. The technique of voltammetry has long been the method of choice for trace metal analysis and works by measuring the current while scanning the entire

voltage range of the solid-state electrode (I versus E curves). This then allows the measurement of more than one species at a given time in the same region of space [BRE 95]. The technique of voltammetry can be viewed as being analogous to absorbance versus wavelength curves in spectroscopy because the electrode currents measured for peaks during voltage scans are proportional to the concentration(s) of the analyte(s). Voltammetry is thus a non-selective method that can measure many chemical species, which over the last two decades has included the application to a suite of trace metals, including cadmium, copper, lead and zinc.

Usually, the monitoring of trace metals has relied on spot sampling and subsequent laboratory analysis using various techniques (see Chapter 4), the major drawback of which is that they measure only total concentrations of the metal present, although voltammetry methods, such as anodic stripping voltammetry and competitive ligand equilibration–adsorptive cathodic stripping voltammetry, have been used for over 25 years for determining both total dissolved metals and for understanding metal speciation [FLO 77, SIP 77, VAN 84]. In voltammetry, a reference electrode (e.g. Ag/AgCl) is used to apply a voltage against a working electrode (e.g. Au/Hg), whereas a counter platinum electrode is used to measure current so that no current passes through the reference electrode. Generally, measurements of total metal concentrations are made after sample acidification and UV irradiation to destroy (complexing) organic matter. Speciation measurements include the determination of complexation properties, such as stability constants and total ligand concentrations, of model or naturally occurring ligands. These are usually obtained by recording the voltammetric peak current intensity of the test metal during the titration of the complexing solution with the metal. The major limitations of conventional approaches to trace metal analyses, even for in-field measurements, are the sample perturbations due to sampling, and possible sample storage and handling effects, and the long analysis times required for metal speciation procedures. The species distribution and properties must be preserved in order to interpret the role of metals in biogeochemical processes, and in particular to assess their ecotoxicological effect. The conventional

laboratory-based techniques were adapted [BUF 05] to work in the field (e.g. on river lake or sea shore) [HUA 99] or on board a ship [ACH 94, WHI 98] to minimize problems encountered with sample storage.

It is now well accepted [BUF 05, TER 08] that the development of rugged, submersible non- or little perturbing devices and probes, for automatic *in situ* measurements (i.e. measurements performed inside the water and sediment/soil matrix), is an important need. Voltammetry is very well suited to this purpose since it can be used in low-cost, automated and miniaturized equipment with low energy requirements. However, significant technical developments are required to improve conventional voltammetric devices before they can be deployed truly *in situ* rather than being limited to use as on-board analyzers. A review of these developments is given in [BUF 05], while an initial overview of the concept of *in situ* voltammetry is given in [BUF 00].

Difficulties in calibration and validation are limitations to the use of *in situ* electrochemical devices. As the systems are measuring in real time, it is difficult to calibrate the instrument *in situ* without undertaking a large number of spot samples for verification. One such intercomparison was used to validate *in situ* voltammetric profiling systems (VIPs). Reliability and performance were assessed in the laboratory using five replicates [BRA 09]. Concentrations of the dynamic metal fractions (cadmium and copper) measured by the VIPs in CRMs were close to the stated values. There was good agreement between the concentrations measured by five VIPs simultaneously deployed for periods of several hours in coastal waters. However, there is still a need for more intercalibration and validation studies in order to increase confidence in the reliability and robustness of these arrays of *in situ* devices. New developments such as environmentally friendly disposable probes with microfabricated bismuth working electrodes for trace metal analysis [ZOU 08], cobalt-based microelectrodes for phosphate [LEE 09] and competitive screen-printed electrode sensors [ZAO 09, ZAO 10] are still in the laboratory development phase; it will be a challenge to adapt them for *in situ* operation.

3.2.6. Sensors

A sensor is a device that produces a measurable response to a change in a physical condition, such as temperature or thermal conductivity, or to a change in chemical concentration. Sensors are ideally suited for making *in situ* measurements in the marine environment and may help overcome some of the current problems of the undersampling (in both space and time) of coastal waters and the deep ocean. Many platforms are available on which these sensors can be deployed, e.g. gliders, benthic landers and moorings (see [GRE 11]). All of these have the capability to address the current data shortage; however, this will be achieved only when biogeochemical sensor systems have undergone a multiple order of magnitude change in size, power consumption, reliability and robustness [DAL 04]. Various types of sensors are reported in detail by Greenwood *et al.* [GRE 11]; the main categories of which are as follows:

– *Physical-and chemical-based sensors:* *In situ* sensors have been used for a number of years to measure physical-based parameters such as conductivity, oxygen, pH and carbon dioxide in seawater. The majority of these are available commercially, particularly in sensor packages where the data can be used to calculate salinity, density, sound velocity and other parameters of interest. Auxiliary sensors are designed to measure other parameters (such as dissolved oxygen, pH, turbidity, chlorophyll *a*, rhodamine, blue-green algae, ammonia and nitrate) along with a device to collect water samples for subsequent analysis in the laboratory. Often arrays of sensors are used so that multiple parameters can be measured simultaneously, and these systems can be fully automated. These can either be deployed from a ship in profiling mode or can be deployed on a mooring for long-term monitoring as part of an observation system. Commercially available conductivity sensors use an AC voltage applied to nickel electrodes or graphite electrodes [OWE 09]. Oxygen sensors are also available commercially for a number of applications, such as profiling sediment–water interfaces, and water columns, based on either electrochemically- or optically-based systems [GLU 00]. Numerous sensors are available (both commercially and as research devices) for

the measurement of pH and pCO₂, parameters that are important in climate change studies [SAB 04, THE 05] and therefore need to be measured accurately in monitoring programs, ideally by *in situ* measurements. The sensors include electrochemical, potentiometric, photometric and fiber opticbased instruments [CAI 00].

– *Biosensors*: Biosensors are devices that integrate a biological recognition element with a transducer of some form that converts changes associated with interaction between the receptor and the target compound into an output signal that can be measured quantitatively. Several reviews [RON 07, FAR 09a, FAR 09b, ROI 09] have provided useful definitions and descriptions of biosensors, and have differentiated them from biological systems (bioassays, biological early warning), where such an integral transduction mechanism is absent. Authors have classified biosensors in a number of ways, usually on the basis of the type of recognition unit or the nature of the transducer. For instance, the biological component can be provided by DNA, enzymes, immunological systems, receptor proteins and whole cells. The transduction component can be provided by acoustic, chemical, electrochemical (potentiometric, amperometric, conductimetric), microbalance, optical (absorbance, bioluminescence, CL, total internal reflection, surface plasmon resonance) and piezoelectric mechanisms. The advantages of biosensors, such as high specificity, sensitivity and, in some cases, biological relevance, and an ability to work in a wide range of matrices make them potentially very useful in the area of environmental monitoring. The use of such devices that could be deployed and operate remotely to make repeated *in situ* measurements that could be transmitted to a laboratory is seen as the ideal way of meeting the requirements for monitoring the quality of environmental water. However, so far there are few commercially available biosensors for use in the environment, and even fewer that can be used *in situ*. There is currently a large gap between the research output, and the commercial availability of appropriate instruments for environmental applications. Progress has been made with biosensors for heavy metals, and some of the key organic pollutants (e.g. herbicides, fungicides, organotins, PAHs, PCBs, and benzene, toluene, ethylbenzene and xylene isomers

(BTEx) components) of concern. Major public investment over many years in Europe has resulted in the development of many potentially useful biosensors [FAR 09]. Although a requirement for specific sensors for measuring individual pollutants has been stated by many authors, there remains a gap between the production of promising prototypes that will achieve this and the delivery of commercially available robust instruments. In part, this is due to the potentially small market for such devices and the high costs of the development to manufacturing stages and validation for *in situ* environmental use. This contrasts with the successful development of commercial instruments based on biosensors for use in medical applications. An important environmental role for biosensors might be in the *in situ* measurement of general water quality (measured as levels of nutrients that can lead to eutrophication, biological oxygen demand), sublethal effects (e.g. endocrine disruption) or general toxicity of the complex mixtures of toxicants that tend to be present in environmental waters [ROD 05]. Another function where development of biosensors might be economically favorable is detection of toxins produced by dinoflagellates and algae that can cause commercially relevant damage that affects producers of some sea foods. Here, they could provide alarm systems that would be widely used and would justify the high development costs. Although highly desirable, and very sensitive for a range of marine toxins, none of the biosensors is available as a commercial instrument for use in food or environmental laboratories. However, devices that can be used to detect algal species that produce the toxins of concern have shown considerable promise and may be closer to commercial availability. Identification of harmful species using microscopical methods is difficult, and methods that can differentiate between harmful and non-harmful species without the need for long taxonomic training and experience would be very helpful. Work has been carried out to develop biosensors for measuring endocrine disruptors [XIO 09]. However, most of systems are a long way from in field, or *in situ* deployment for monitoring endocrine disruptors in environmental water. Finally, biosensors offer the potential to replace the use of bioassays and to provide rapid, automated *in situ* monitoring of water quality. Several strategies have

been used to develop biosensor systems that can provide rapid, sensitive measurements of the toxicological quality of environmental waters, e.g. with an array of whole cell sensors that respond to a range of toxic insults and signal damage to different cellular systems [LEE 05]. The use of biosensors for the measurement of general toxicity is a desirable goal, and would significantly enhance the ability of regulators to monitor water quality in a way that would provide early warnings of problems and timely information on the results of remediation actions. It would also allow a better regulation of discharges than is possible with infrequent spot sampling and chemical analysis for a limited number of compounds of interest that provides no information on the interactions that can occur between toxicants. However, currently there are no biosensor technologies available that could be applied *in situ* in the marine environment.

3.2.7. Biological early warning systems

In contrast with most of the devices discussed in this chapter, biological early warning systems (BEWSs) are used to detect events like (accidental) spills, and not to detect the average condition of the aquatic environment. They make use of living organisms to detect incidental pollution. Organisms can “smell” the rapid increase in the occurrence of toxic chemicals. They react swiftly, so that an alarm can be generated to warn of, for example, an accidental spill. The rationale behind their use was that routine monitoring programs in river/seawater chemically analyze only a small part of the large number of chemicals that can potentially enter the aquatic environment, either by discharge or accidental spill. Organisms may detect and sense many more compounds than those routinely analyzed chemically. Although they will certainly not detect all potentially dangerous compounds, aquatic organisms can be used as wide spectrum sensors. In addition, where as most chemical monitoring is based on spot samples collected at weekly intervals (or even lower frequencies), organisms work around the clock, 7 days per week. When an alarm is generated by the BEWS, it may be used to trigger automated sampling so that chemical analyses can be used to find evidence of the causative compound(s), although it is not always

possible to define the cause of even a clear biological effect. With the exception of some highly specific biosensors described above [GRE 11], organisms in a BEWS are not able to define the nature of the chemical(s). A BEWS is a semi-quantitative sensor, not a qualitative detector.

In order to obtain an early warning, it is necessary to use organisms that can give a rapid biological response (alarm) to a relatively steep increase in the concentration of toxic pollutants within a period of minutes to hours. Unlike bioaccumulation studies such as the “mussel watch”, originally proposed by Goldberg [GOL 75], where it may take several weeks to reach an equilibrium between water and tissue concentration, the response needs to be fast and the biological functions monitored need to change rapidly following changes in the environment such that there is a functional and preferably quantifiable reaction to toxic chemicals in the water column. In practice, this means the detection of changes in either behavior or in physiological parameters. Behavioral responses that can be used in BEWS include activity, locomotory behavior, avoidance, positive rheotaxis and escape behavior [DIA 88, KRA 91, GER 06]. Physiological responses potentially include respiration rate, ventilation frequency (fish), heart rate, pumping rate, bioelectric potential, photosynthesis and growth rate, bioluminescence, ion flux, blood chemistry and hematology, and death [KRA 91, HAN 99]. Irrespective of which biological function is used, BEWS are based on the detection of an “abnormal” situation. The current behavior/physiological status will always be compared with a past status, either by using a calibration curve or threshold, or (more commonly) from historic recording of the organisms’ own functioning (e.g. of 1 h before). Comparison with a control system is useful in the laboratory, but cumbersome in a field situation. Once an abnormal situation is detected with sufficient confidence, an alarm is generated by the system.

Requirements for a successful BEWS are a combination and integration of a suitable (preferably endemic) test organism, a method of detection of changes in physiology or behavior and a means of

signal processing, data evaluation and alarm generation. It will be obvious that a biological system can be used only when the conditions of the ambient water does not prevent normal life of the organism. Therefore, the temperature, oxygen content, pH, salt content and the background toxicity should be appropriate for the organisms used. Furthermore, the successful BEWS should be easy to operate, the organisms easy to obtain, the system reliable and capable of working for weeks to months with the least amount of false alarms [BAL 94]. The system must be robust and fully validated for field application. Too often a monitoring system works well in the laboratory, but fails in environmental application [GRU 94, VON 98]. Finally, costs, notably of maintenance (e.g. frequency of replacement of organisms), will also determine its success.

Most commercial BEWS systems that have passed the laboratory phase and are implemented in the field have been developed for monitoring fresh water/drinking water [KRA 91, GER 06, KRA 09]. Typical organisms and associated alarm responses used in practical applications include several types of fish (ventilation frequency, locomotory behavior and positive rheotaxis), bivalves (valve movement), small invertebrates, e.g. *Corophium sp.* (locomotory behavior), algae (fluorescence) and bacteria (various physiological parameters, including luminescence). Typical applications have been discussed by Kramer [KRA 09] for several case studies.

Application in the marine environment is hampered by the limitation that many of the organisms most commonly used in BEWS cannot survive in seawater. Without aiming to provide an exhaustive account, several systems have been demonstrated to work successfully in the marine environment; others may be easily adapted for use with marine species.

For some BEWS, guidelines and validation protocols have been developed [KRA 09]. These guidance documents detail the necessary experiments and provide a good basis for the validation of BEWS in monitoring practice (e.g. [WAG 08]). For spot sampling and subsequent laboratory analysis, compounds can both be

identified and quantified. In contrast, BEWS can in principle only be semi-quantitative and offer no identification. Kramer [KRA 09] argued that validation methods that are tuned to the test system principles that take into account the level of identification and quantification should be developed.

3.2.8. Future

As the importance of a healthy marine environment has become more widely recognized, and legislation is being extended to provide protection, the need for reliable and widespread monitoring becomes more urgent. The logistic problems associated with representative sampling over wide areas preclude the use of strategies based on spot sampling that were developed for monitoring fresh water. New strategies need to be developed, and although the task is daunting, significant progress has been made in devising methods that can be used to collect information remotely. However, there are still barriers to be overcome before long-term remote deployment can become routine. While many successful deployment devices (including Argo floats, landers, auto-submarine vehicles and smart buoys) and associated communication systems are available, the range of routine measuring devices to use with them is relatively limited.

Biofouling of instruments, optical and sensor surfaces is among the problems to be solved. This is a challenge in all aquatic environments but is particularly marked in nutrient-rich coastal waters. Approaches that have been used include mechanical scrapers, non-toxic (e.g. silicone greases) and toxic (e.g. TBT) barriers, and the use of copper coatings, meshes and shutters. Other solutions are being sought in the use of coatings that peel away over a deployment period and biomimetic surfaces (e.g. peptide-peptoid conjugates, and phosphorylcholine-based polymers) that were initially developed for medical applications.

Operational field lifetimes of remotely deployed instruments are currently limited by the power consumption of instruments and the available power supplies. Cabled observatories can provide the power

to operate sensor networks for extended periods; however, the establishment of the required infrastructure is expensive and, by necessity, limited in scope. Many *in situ* sensors still rely on commercially available batteries. The use of renewable energy systems that can utilize naturally occurring chemicals and conditions in the water body are potential options that hold some promise for the future. These include use of methane hydrate fuel cells, microbial fuel cells, sea-surface photovoltaic cells and motion-to-electricity conversion techniques.

Recent developments in microfabrication, microfluidics and integrated optics in many areas of analytical chemistry are being applied in the development of *in situ* monitoring devices for the marine environment. Lab-on-a-chip technologies have significant advantages in terms of a small size and limited reagent and power requirements. The challenge is to ensure that these systems can attain the sensitivity needed for many marine applications where analytes are present at only trace concentrations. However, as the overall cost of the sensing system is reduced, this would potentially enable the deployment of larger numbers of devices and thereby improve the spatial and temporal resolution and extent of offshore monitoring activities.

Currently, there is a lack of recognized validation protocols for many of the methods described in this chapter, and a lack of support networks for QA and QC. The commercial development and wide acceptance of *in situ* technologies will depend on the availability of systems such as those available for classical, laboratory-based instrumental analytical procedures. There is a need for innovations in the development of suitable reference materials and interlaboratory trials.

Most of the devices with the potential for use in the *in situ* monitoring of marine waters were developed for use in the laboratory where they could serve as less expensive, more rapid and more sensitive replacements for conventional analytical methods. They could enable frequent screening of water quality to avoid the problems

associated with infrequent regulatory sampling. The more expensive regulatory measurements could then be used only when required. However, much development and validation work is necessary to reach this goal. The long-term aim of using these technologies for *in situ* monitoring in the marine environment is even further away. Although many devices have been developed to proof of concept or prototype stage, the development work to convert these into robust, reliable, validated instruments that can be deployed in the marine environment for prolonged periods (weeks to months) has not been undertaken. This may be a consequence of the short-term nature of research funding, and the failure to provide associated support for the development work necessary to take advantage of exciting research innovations; there have been many lost opportunities by failure to capitalize on significant research developments.

3.3. Biomonitoring

3.3.1. Introduction

Biomonitoring objectives, approaches and techniques have been reviewed by Haarich [HAA 11]. It is associated with different fields of investigations on biological material; in particular, one main task within biological monitoring programs is the investigation of organisms with respect to abundance, condition, age distribution and other biological parameters related to biodiversity like the composition of species to detect changes in marine habitats. The chemical analysis of marine organisms is another well-established part of marine monitoring programs, using biota as suitable matrix to detect and quantify the presence and concentration of hazardous substances. A more recent kind of biomonitoring is the investigation of biological effects (hereafter referred to as bioeffects), using cellular and molecular tests determining diseases or using histopathological methods, with the aim to detect effects of natural and anthropogenic impacts on the health status of biota. The ideal case would be a definite correlation between a hazardous substance, its biological effect and implications on the reproduction of marine organisms, so

that all mentioned kinds of marine biomonitoring will form a closed cause–effect chain.

In the past 15 years, the co-operation between scientists performing chemical monitoring and those investigating biological effects in marine biota has been developed to integrate chemical and bioeffect monitoring. Therefore, this chapter will focus on the chemical monitoring of hazardous substances in marine biota, but will also include aspects of the integration of chemical and bioeffect methods [HAA 11].

One of the reasons to use biota for environmental chemical monitoring is that organisms are accumulating some metals, metalloids and many persistent lipophilic substances that are known to be toxic, carcinogenic or possessing endocrine disrupting properties. As concentrations of hazardous substances are generally relatively low in the marine environment due to the large dilution when entering the sea from the rivers, local sources and the atmosphere, it is more likely to be able to detect and quantify them in biological tissues due to the bioaccumulation. Less polar, hydrophobic substances are therefore mainly analyzed in biological matrices, even if they are still detectable in sediment or water, because of the higher concentrations due to bioaccumulation.

Another advantage is that the accumulation period is restricted to the age of the organism. Particularly for young individuals and females, which are releasing part of the contaminants by spawning, the monitoring results are representative for the level of exposure of the recent period. This is, amongst others, of interest for assessing the effects of reduction measurements or the consequences of accidents of bigger amounts of chemicals entering the marine environment. An aspect that is obvious, but often not mentioned, is that the organisms are the living part of the marine environment, which is impacted directly and negatively by hazardous substances, so that monitoring them serves as an indicator for the contamination status of the environment as well as of the investigated biota itself.

Typical biota used for monitoring purposes are fish and shellfish. For fish, liver or muscle tissue is analyzed, depending on the substance.

For shellfish, the soft body is used for analysis. The analysis of eggs from seabirds that are feeding at sea has also proven to be a reliable way of monitoring certain regions of the marine environment, determined by the feeding area, particularly as seabirds represent the top predator level (see references under “seabird eggs” in section 3.1.3).

3.3.2. Analytical trends in chemical monitoring of marine biota

For a long time, a selection of organochlorine compounds (PCBs, DDTs and HCHs) and some heavy metals (Cd, Pb, Hg, Cu and Zn) has been typical for chemical marine monitoring. Chlorinated dioxins and furans are also of high importance, but normally not part of regular environmental marine monitoring due to the high analytical effort; however, they are the subject of investigations on seafood for human consumption. The monitoring of radionuclides (Cs-137, Sr-90, Pu-238, Pu-239 240, Am-241) is usually organized in separate programs [HAA 11].

Analytes					
					Methodological Guidance
Heavy metals and metaloids	Cd, Pb, Cu, Zn,				OSPAR 2009
	Hg				OSPAR 2009
Organometallic compounds	Methyl-mercury				
	Organotin compounds	Tributyltin (TBT); triphenyltin (TPhT)	Fish muscle	GC-MS, GC-FPD, GC-ICP-MS; HPLC	OSPAR 2009

Table 3.3. *List of metals being of interest to be analyzed in marine biota (after [HAA 11])*

Particularly for organic substances, only a very small selection of thousands of compounds entering the sea can be measured and is represented in the regular programs, and therefore current monitoring efforts do not cover well the actual situation of the presence of organic contaminants. A range of additional compounds, which are likely to enter the sea due to their production, use and physicochemical properties, has been investigated on their presence in the marine environment by a directed analysis. These candidate substances are published in so-called priority substances lists. They are the subject of projects and research, one-off surveys or target screening programs being performed at intervals of several years. Data on other compounds can also be obtained by means of undirected analytical methods, the so-called non-target screening. The moment such a compound is discovered to be present in the marine environment and that such information can become relevant for possible further action, we need not to correspond to a recent input into marine waters; in many cases, the improvement of analytical methods and instruments has enabled to detect and quantify substances that are produced and used in high quantities since many years, for example the brominated flame retardants, chlorinated paraffins and perfluorinated compounds (PFCs).

Analytes					
					Methodological Guidance
Alkylated phenols		Nonylphenols (NP); nonylphenoethoxylates (NPE); octylphenols (OP); octylphenol ethoylates (OPE)	Fish muscle		
Brominated flame retardants	Polybrominated dipenylether (PBDEs)	BDE 47, 85, 99, 100, 153, 154	Fish muscle, fish liver	GC-ECD, GC-MS,	OSPAR 2009
	Hexabromocyclododecane	HBCDD	Fish muscle		OSPAR 2009
	Tetrabromobishenol-A	TBBPA			
Chlorinated organic compounds	chloroalkanes	Short-chain chlorinated paraffins (SCCPs, C10-C13); medium-chain chlorinated paraffins (MCCPs, C14-C17)	Fish liver ¹	GC-MS	Oehme

	Chlordanes	<i>cis</i> -chlordane, <i>trans</i> -chlordane, <i>trans</i> -nonachlor	Fish liver ¹	GC- ECD, GC-MS	Oehme
	Dibenzodioxines, furans and co-planar CBs	PCDD/Fs		HRGC/ HRMS	MCWG 2009
		DDE, DDD, DDT	Fish liver ¹	GC- ECD, GC-MS	OSPAR 2009
	Hexachlorobenzene	HCB	Fish liver ¹	GC- ECD, GC-MS	OSPAR 2009
	Hexachloro- cyclohexanes	a-HCH, b-HCH, g-HCH, d-HCH	Fish liver ¹	GC- ECD, GC-MS	OSPAR 2009
	Polychlorinated biphenyls	PCBs	Fish liver ¹	GC- ECD, GC-MS	MCWG 2009
		Endosulfan		GC- ECD, GC-MS	Theobald
Perfluorinated compounds (PFCs)		Perfluorooctane sulfonate (PFOS); perfluorooctanoic acid (PFOA)	Fish liver ¹	LC-MS	Theobald OSPAR 2009
Polyaromatic hydrocarbons (PAHs)			Biota	GC-MS, HPLC- UVF	OSPAR 2009
		PAH metabolites	Fish bile	HPLC- UVF	Kammann
Alkylated PAH			Biota		OSPAR 2009

¹ Muscle of higher lipid content; when analyzing for human consumption, the edible part is preferred.

Table 3.4. *List of organic substances being of interest to be analyzed in marine biota (according to [HAA 11])*

For most of these compounds, the analytical details are described in the Technical Annexes to the OSPAR-JAMP Guidelines for Monitoring Contaminants in Biota [OSP 09], which have been updated and supplemented in the past years by the ICES Marine Chemistry Group (MCWG).

Although volatile organic compounds have been detected in marine biota, in coastal areas often with higher concentrations of many priority substances ($\mu\text{g/kg}$ level), they have not become part of the marine monitoring programs, obviously because of their low potential to bioconcentrate and accumulate [ROO 05].

Compound/group	Combine indicator species	BSAP indicator species
DDT and metabolites	Herring/perch muscle (core/coastal core program)	
	Cod liver (main program)	
	Macoma baltica soft tissue (main program)	
	Blue mussel soft tissue (supporting program)	
CBs (nos. 28, 52, 101, 118, 138, 153, and 180)	Herring/perch muscle (core/coastal core program)	
	Cod liver (main program)	
	Macoma baltica soft tissue (main program)	
	Blue mussel soft tissue (supporting program)	
Dioxins (PCDD), furans (PCDF) and dioxin-like polychlorinated biphenyls		In fish (herring or salmon or perch) muscle
Hexachloro-benzene (HCB)	Herring/perch muscle (core/coastal core program)	
	Cod liver (main program)	
α - and γ -hexachloro-cyclohexane (HCH)	Herring/perch muscle (core/coastal core program)	
	Cod liver (main program)	
	Macoma baltica soft tissue (main program)	
	Blue mussel soft tissue (supporting program)	
Chlorinated paraffins (SCCP, MCCP)		Substance of concern, but no BSAP indicator
Endosulfan		Substance of concern, but no BSAP indicator
TBT	TBT in <i>Mytilus edulis</i> soft tissue (coastal supporting program)	
	Imposex in whelk Buccinum (coastal supporting program)	
Brominated flame-retardants PBDEs	In herring tissue not specified (supporting program)	Substance of concern, but no BSAP indicator
Brominated flame-retardants other	Herring tissue not specified (supporting program)	Substance of concern, but no BSAP indicator

Perfluorinated compounds (PFOS, PFOA)		PFOS in sediment or fish (species optional) liver
Organotin compounds (TBT, TPhT)		TBT in sediment or biota (fish or mussel) or imposex
Alkylated phenols		Substance of concern, but no BSAP indicator
Compound/group	Combine indicator species	BSAP indicator species
Mercury, Hg	Herring/perch/flounder muscle (core/coastal core/coastal main program)	In fish (herring or flounder or perch) muscle
	Cod muscle (main program)	In bivalve (blue mussel or Baltic clam) soft tissue
	Macoma baltica soft tissue (main program)	In fish (herring or flounder or perch) muscle/edible part
	<i>Mytilus edulis</i> soft tissue (coastal core program)	
	Herring tissue not specified (supporting program) different age classes	
Cadmium, Cd	Herring/perch/flounder liver (core/coastal core/coastal main program)	In fish (herring or flounder or perch) liver
	Cod liver (main program)	In bivalve (blue mussel or Baltic clam) soft tissue
	Macoma baltica soft tissue (main program)	In fish (herring or flounder or perch) muscle/edible part
	<i>Mytilus edulis</i> soft tissue (coastal core program)	
	Herring tissue not specified (supporting program) different age classes	
Lead, Pb	Herring/perch liver (core/coastal core program)	
	Cod liver (main program)	
	Macoma baltica soft tissue (main program)	
	<i>Mytilus edulis</i> soft tissue (coastal core program)	
	Herring tissue not specified (supporting program) different age classes	
Copper, Cu	Herring/perch liver (core/coastal core program)	
	Cod liver (main program)	

	Macoma baltica soft tissue (main program)	
	Mytilus edulis soft tissue (coastal core program)	
	Herring tissue not specified (supporting program) different age classes	
Zinc, Zn	Herring/perch liver (core/coastal core program)	
	Cod liver (main program)	
	Mytilus edulis soft tissue (coastal core program)	
Caesium-137	Obligatroy in fish (species depending on catch composition and amounts per species)	In herring muscle as indicator for whole Baltic Sea In plaice and flounder muscle for Southern Baltic Sea (southwards from Gotland)

Table 3.5. Biota monitoring in the Baltic Sea
(Combine and BSAP)(according to [HAA 11])

3.3.3. Main features of biota monitoring programs

3.3.3.1. Sampling

General information about the objectives of a monitoring programme and the conditions for temporal trend or spatial monitoring have an influence on the way sampling of marine biota is designed. Most often, this information includes the sampling frequency and the spatial coverage, depending on what should be assessed and which level of detail is needed. Regarding the sampling frequency for biota monitoring for long-term temporal trend assessments, at least one sampling period per year is common. As the condition of animals is changing periodically over the year (biological cycle), sampling typically takes place in a stable period regarding environmental conditions and physiological processes, in any case outside the spawning period. This period will differ for species and area. For example, for fish in northern European seas, this period is

summer to autumn and for mussels, this period is late autumn to winter [HAA 11].

3.3.3.2. *Choice of species and tissue*

The choice of species for biomonitoring depends on the specific objectives of the monitoring program and can only be determined on the basis of information on the sampling area, the composition of the stock or population of the fish, shellfish or seabirds and their migration pattern. The species should be representative and sufficiently abundant, ideally throughout the entire monitoring area, and the tendency to migrate should be low, so that results can be related to the surrounding environment.

With regard to the selection of the species to be monitored for chemical contaminants, we should take account of the following requirements: the species should accumulate the contaminant in sufficient amounts without being seriously affected; the specimens collected should be of a reasonable size, giving adequate amounts of tissue for chemical analysis, and if part of an integrated program, also for biochemical and physiological analyses; shellfish should be hardy enough to survive for defecation before being prepared for analysis.

The choice of tissue is predominantly determined by the degree of bioaccumulation and the related concentration of the contaminant, so that for most species the liver is preferred for lipophilic compounds. For fatty fish, with relatively high lipid content in the fillet, it may be of advantage to use the muscle tissue instead of the liver, even if the concentrations are lower, as in most cases, the lower signal height in the chromatogram of the muscle tissue sample is compensated by a lower influence of the matrix, thus resulting in a better signal-to-noise ratio and a better peak separation. The decision to analyze individual or pooled samples also depends on the amount of tissue available from the individual sample of a given age, size and gender. For example in the case of fish, there could be a conflict between the need to get sufficient amount of material and (i) the objective to monitor the recent period and therefore preferring younger and smaller fish, (ii) to analyze on an individual basis to be able to calculate the within-batch/-catch variation for temporal trend monitoring and (iii) to

analyze chemical and biochemical parameters/biomarkers as much as possible from the same individual sample for integrated chemical and bioeffects monitoring purposes.

3.3.3.3. Choice of size, age, sex and number of individuals

When selecting samples from a catch, it is normally not possible to determine the age. As size and age of fish and shellfish are correlated rather well for most species, the length of the fish or the shell can be used as a selection criterion. The age of the fish should be controlled by analysis of the otoliths at a later time. For freshly caught flatfish, the sex can be determined without dissection by screening the shape of the gonads shining through when illuminated from the back.

Where it has been successfully applied in the past, length-stratified sampling may be maintained. For this procedure, the length range is divided in five equal groups on a logarithmic scale; for each length interval, at least five individuals have to be selected. For some areas, the length range has become smaller, due to changes in the composition of the population, particularly for fish, so that data obtained by this procedure were no longer comparable. As the age to length correlation and the contaminant load differ between male and female individuals, it is often more appropriate to sample with a view to minimize natural variability within the sample. This can be done by only selecting individuals of the same sex and a narrow length range reflecting younger fish, e.g. only young females. This causes a significant reduction in the within-batch variability, and particularly for time trend assessments, more reliable statistical results can be obtained from shorter time series.

For shellfish, a sample should be collected with the number of individuals large enough to be divided into at least three equal pools with each pool consisting of at least 20 animals and enough soft tissue for all analyses. The length of the individuals collected should be constant from year to year at each station, or should at least fall within a very narrow range, e.g. within 5 mm. To reflect recent levels of contamination, young individuals should be chosen. Examples and more details regarding the described selection criteria are given in the JAMP guidelines [OSP 09].

The minimum number of individuals for the length-stratified sampling is 25, which results in at least five analytical samples when pooled. For individual analysis, statistical calculations of North Sea fish data [NIC 97] recommend a minimum of 12 fish. Compared to 25 analyses, the small loss of statistical significance is justified by the lower analytical extend and the related costs, whereas a significant improvement would need a much higher analytical effort by more than three times the number of samples to be analyzed.

3.3.4. Analytical methods

The main differences in analytical methods concern the sample preparation, extraction and clean-up methods. Therefore, this section mainly deals with the special aspects of biota samples. More details on instrumental determination of micropollutants are given in Chapter 4.

3.3.4.1. Sample preparation

Fish can be dissected for subsampling immediately after being caught, if appropriate conditions are available on board. One of the main conditions that requires particular attention is to avoid contamination of the sample. Shellfish individuals in samples should defecate before used for analysis, which implies that they will not be processed immediately after sampling. Seabird eggs will be opened under clean conditions in the analytical laboratory.

For trace metal analysis, it is necessary to dissect and wrap the tissue sample working under clean conditions to avoid contamination by dust and other particles. On board this can be done in a laminar airflow workstation, a so-called clean bench. If there is no clean bench available or no possibility to install such a device temporarily, the ungutted sample has to be wrapped in bags or foil of appropriate properties (e.g. aluminum foil and polyethylene (PE) bags) and immediately frozen and stored for subsequent treatment later in the land-based laboratory. The ideal solution on board would be to work in a fully equipped cleanroom container.

For organic contaminants, it is possible to dissect them in a laboratory on board, as long as the equipment and the fresh air supply is designed to avoid contamination by exhaust gases or evaporation of volatile organic substances, e.g. from sealing materials or furniture. Good basic conditions are possible when walls and laboratory furniture are manufactured from seawater resistant stainless steel, when the floor is made without softeners and when fresh air is available and not affected by exhaust gases from the engine or the galley.

For dissection of subsamples for trace metal analyses, only metal-free knives, scalpels and tweezers must be used. For organic contaminants, stainless steel or ceramic blades are suitable, but plastic tweezers must be avoided. For cleaning between samples, high-purity water and, for fatty samples, organic solvents (acetone, alcohol or mixtures, at least analytical grade purity) should be used. On board at offshore areas, fresh seawater is also suitable for cleaning the equipment.

Subsamples (e.g. of liver) should be stored in clean containers made of glass (e.g. borosilicate glass), stainless steel or aluminum, or should be wrapped in clean aluminum foil and frozen quickly in liquid nitrogen or in a blast-freezer. The individual samples should be clearly labeled and stored together in a suitable container placed in a deep freeze at $<-20^{\circ}\text{C}$, until analysis. Subsamples for enzyme tests should be stored in vials suitable for storage in liquid nitrogen, labeled clearly and stored in liquid nitrogen until analysis.

Further details are given, for example, in Chapters 5 and 6 of the JAMP Guidelines for Monitoring Contaminants in Biota [OSP 09].

3.3.4.2. *Extraction and cleanup*

For metals, the classical procedure is to freeze-dry and homogenize the tissue, followed by digestion with strong acids. For very low concentrations, a complexation and re-extraction step to reduce the matrix is recommended when determined by Graphite Furnace Atomic Absorption Spectrometry (GFAAS). Although procedures are described in the literature about digestion in open vessels, it is

recommended to perform digestion under pressure using quartz tubes heated in closed steel cartridges on a heating block or in closed Teflon vessels in a microwave oven; the latter is nowadays the preferred method due to shorter digestion times and an easier and safer handling. The classical determination is by atomic absorption spectroscopy with the graphite furnace technique (GFAAS), which is more and more substituted by multielement methods based on inductively coupled plasma (ICP) ionization and spectrometric detection like MS or optical emission spectroscopy. A lesser used applied method is the total reflection X-ray analysis, also capable of multielement determination in low concentrated environmental samples.

For mercury analysis, instruments for the direct determination from the solid or liquid sample without any preceding digestion have been recently developed, which may substitute the common methods of digestion, reduction to elemental mercury by Sn(II) or hydride generation, followed by amalgamation where appropriate. The determination is performed by cold vapor atomic absorption spectroscopy (CVAAS) or cold vapor atomic fluorescence spectroscopy (CVAFS). These methods are applied for measuring the so-called total mercury. To distinguish between inorganic mercury and organic methyl- and ethyl-mercury compounds, a chromatographic separation is needed. For gas chromatographic separation, the compounds have to be alkylated to become volatile. The alkylated compounds have to be decomposed in a thermic reactor to generate elemental mercury when using AAS or AFS for the detection. A literature research is recommended to find the best combination of existing modifications for extraction, formation of intermediates and detection methods for the material to be analyzed and for the currently available equipment, as those are specifications that cannot be described in detail here.

For organic contaminants, the entire analytical procedure has to avoid any losses of target analytes by vaporization or deterioration. In a first step, the tissue has to be dried and homogenized. Classical procedures are also freeze-drying with subsequent cryo-milling or milling with water-free sodium sulfate and quartz sand in a mortar

grinder, or cutting the tissue mechanically and mixing it with quartz sand and drying the relatively coarse homogenized sample in a common microwave to remove the moisture [HAA 11]. Lipophilic analytes will be extracted together with the lipids by solvent extraction, which may be performed by column extraction at normal pressure and room temperature (cold extraction), with Soxhlet extraction under reflux or automated by Accelerated Solvent Extraction (ASE by Dionex[®]) under pressure at temperatures above the boiling point of the used solvent. Using ASE saves time and solvent and provides a better reproducibility. The necessary reduction of the solvent volume is performed using rotating evaporators or multisample evaporators (e.g. by Buechi[®]), which may be adapted regarding the tubes to the preceding extraction device, so that a change in the tube is no longer necessary. Column chromatography is applied to separate the analytes from the lipids, by adsorption chromatography with lipid retaining fixed phase (e.g. Florisil[®]) or gel permeation chromatography (GPC) using the principle of size exclusion to extract the lipids in front of the analytes. GPC has also the advantage that it is automated to process the samples mostly unattended and also over night to save time. The final step before measurement is the clean-up by chromatography (e.g. activated silica), when necessary, combined with fractionation by gradient solvent elution. However, even after this procedure, the quality of the chromatograms may be not sufficient due to matrix components, which behave very similar to the target compounds and which were not removed before, making it difficult or impossible to fit the signals, when using GC-ECD detection, due to a disturbed baseline. If we can relinquish on certain sensitive compounds such as dieldrin, a further extraction with concentrated sulfuric acid may be successful. Otherwise, cleaning-up needs some experiments to find a suitable chromatographic phase that is able to remove the disturbing substances, and which has sufficient purity for trace analysis purposes. A general recommendation cannot be given. Using a GC-MS system will reduce this problem; nevertheless, also here a sufficient chromatographic separation should be aspired.

The described methods cannot be applied without major modifications for the more polar PFCs such as perfluoro octanoic acid

(PFOA) and PFOS, which are typically determined by LC coupled to tandem MS (LC-MS (-MS)). Although methods are described in the literature for biological tissue without removing the lipid and other matrix components to a major extent, for marine samples clean solutions are necessary to achieve low detection limits and to avoid fast soiling of the LC/MS (-MS) equipment. To avoid soiling, biota samples can be extracted with alcohol, and the lipid is removed by sequential freezing, followed by SPE cleanup [THE 07].

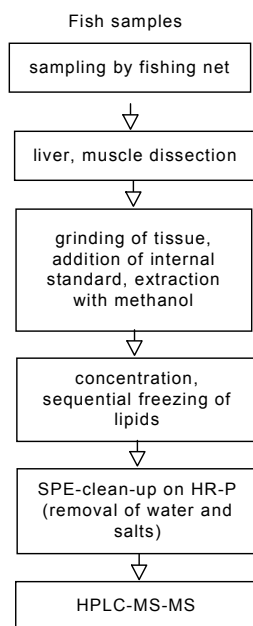


Figure 3.4. Analytical procedure for the determination of PFCs in fish tissue samples (modified from [THE 07])

3.3.4.3. Detection and quantification

The final instrumental measurement of biological samples, as a part of the overall analytical procedure, does not differ significantly from that applied for water or sediment samples with respect to the instrumental equipment, the use of internal standards, calibration and quantification. The determination can be performed by GC-ECD, but more and more laboratories have changed to mass spectrometric

detection in different modes and instrumental modifications (GC-MS, GC-MS-MS, in electron impact or NCI, PCI mode) or liquid chromatography (HPLC-MS or HPLC-MS-MS). Extensive literature is available for the detailed description of the instrumental parameters, e.g. from instrument manufacturers and column suppliers.

3.3.4.4. *Quality control*

QC requirements are also described in many places, e.g. the guidelines of the regional conventions of HELCOM and OSPAR, and are discussed separately in Chapter 2. One general problem should be mentioned that causes difficulties for laboratories analyzing marine biological samples, particularly for organic compounds at very low concentrations. There exists only a very limited range of CRM appropriate for marine biota monitoring regarding the matrix, the concentration level and the selection of certified compounds. Also the test materials supplied by the organizers of external proficiency testing schemes does often not cover the concentration range detected in the monitoring samples of the individual laboratory, so that conclusion drawn from the results of such tests may not necessarily be transferable to the quality of the monitoring results. As the effort to prepare and certify such materials is high and costly, but the relatively low number of customers, a co-operation on a regional level between customer institutes having facilities for sampling suitable materials and institutions or agencies equipped and experienced to process this material, may reduce this problem. Proficiency testing schemes for external QC for chemicals in biota as well as for bioeffects measurements in the marine area are established on an international level to support the performing institutions (see BEQUALM and QUASIMEME).

3.3.5. *Integration of chemical and biological effect monitoring*

When biological effects measurement techniques were introduced in marine monitoring, one of the main goals of their supporters was to be able to substitute chemical investigations. However, more than a decade of investigations has demonstrated that the majority of

biological effects methods are not specific enough to meet the requirements that would allow this substitution. So a change in the aim has taken place from a substitution approach to complementarity. In this sense, the chemical measurements are to aid the interpretation of the biological effects measurements in terms of identifying the chemical causes of the biological effects and establishing concentration responses. Biological effects measurements will serve for early warning, for integrating effects of complex mixtures (synergism and antagonism), for indicating impacts also when concentrations of each chemical compound separately is below its detection limit, for indicating impacts of novel and often not identified chemicals and for indicating impacts on a cellular or molecular level that may not directly be linked to the measured concentrations of chemicals. Following the strategy of the OSPAR-JAMP, biological effects monitoring in relation to hazardous substances is necessary to “take all possible steps to prevent and eliminate pollution and to take the necessary measures to protect the maritime area against adverse effects of human activities so as to safeguard human health and to conserve marine ecosystems and, when practicable, restore marine areas which have been adversely affected”. Within the European marine strategy framework, most work to promote the integration of contaminant and bioeffects monitoring taking into account the existing knowledge and work of expert groups in both fields (e.g. ICES expert groups) has been done by the four ICES/OPAR workshops on Integrated Monitoring of Contaminants and their Effects in Coastal and Open Sea Areas (WKIMON).

Integration of chemical and bioeffects monitoring implies to take care of several practical aspects when planning and preparing the sampling. The ideal case would be to take subsamples from the same individual fish or mussel for both chemical and bioeffects measurements. However, for this, the available amount of material may not be sufficient (e.g. the liver of a small flatfish) or the pre-treatment procedures could be incompatible for simply dividing the material, e.g. for enzyme test methods and metal analysis, the latter requiring homogenization as the analytes are not evenly distributed in the tissue. More detailed guidelines for contaminant-specific biological effects can be found at OSPAR [OSP 08].

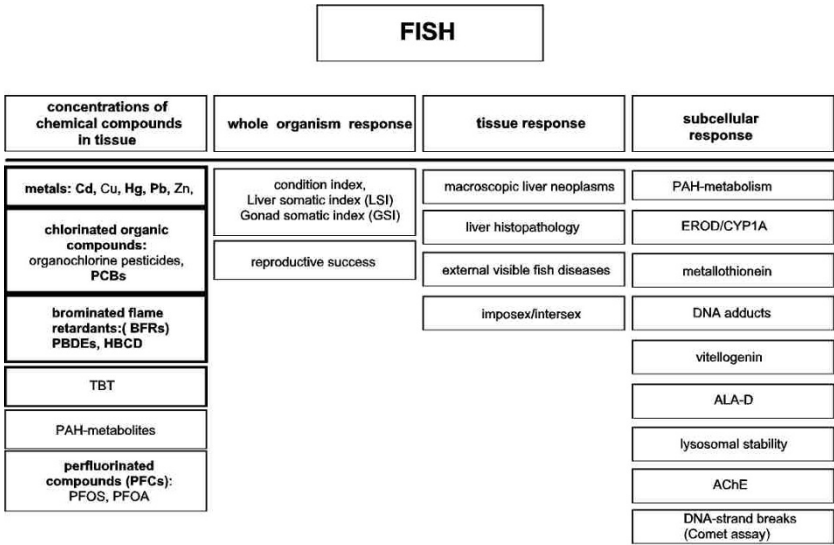


Figure 3.5. Biomonitoring of fish (modified from [WGB 06])

m			Imposex	Gastropods	TBT	
			Intersex	Bivalves	Metals (Pb, Cd, Cu, Zn) butyltin-compounds	Rank, 2009
v	14	s	PAH metabolite	Fish bile	PAHs	
v	15		Bioassays			
v	15		CYP1a			
v	15		Lysosomal stability			
v	15		Liver histopathology/ macroscopic liver neoplasmns	Fish		
v	15		External visible fish diseases	Fish		
v	15		Reproductive success in fish	Fish		

Table 3.6. Biological effects monitoring in OSPAR-CEMP

3.4. Use of sediment in coastal monitoring

3.4.1. *Introduction*

Sediment is an inherent part of aquatic systems, both containing freshwater and seawater. Sediment consists of mineral and organic particles originating from the weathering of rocks, the erosion of soils, river banks and coast, the particulate detritus matter production of organisms and from the wet and dry atmospheric deposition, introduced directly or via surface runoff. The sediment is not a static component of an aquatic system, but the inorganic and organic material is susceptible to transportation [KOW 11]. In the coastal zone, sediment geochemistry is controlled by different natural factors such as hydrology, morphology and oxic conditions. After deposition, the sediment undergoes mineralization, disaggregation and various biogeochemical processes. Changes in flow velocity and direction of the water course result in the resuspension of already settled particles. The fine sediment particles are more susceptible to resuspension than the coarse sediment. As a result, at some distance from the particulate input, a fractionation occurs. In areas of more laminar water movement, the bed contains more fine sediment grains than those of more turbulent flow [OWE 07]. Ions of trace elements and hydrophobic organic compounds, including the most harmful contaminants, usually have greater affinity to fine particles than to the coarse sediment grains. As a result, contaminants concentrate in suspended particulate matter (SPM) and fine-grained sediment fractions, which are more liable to transfer by water than coarse sediment, though also a fractionation of contaminants occurs, depending on particular contaminant affinity to sediment particles [KOW 03, TSA 09, LUB 10]. Sediments are thus depleted in the more hydrophilic compounds, which dissolve better in water and are transported in the dissolved form [OWE 07, ALA 10].

Resuspension of already settled particles in estuaries is caused mainly by river mixing driven by tides. Deltas, estuaries and other coastal regions are often places favorable for human settlement and have become economically important. All the above circumstances

result in intensive municipal, transport and industrial human activities enhancing the contaminant load trapped in sediments and introduced by the river to the sea [MUX 07]. Human activities include those connected with relocation of sediments like dredging to maintain or deepen waterways and harbor basins, or hydromorphological changes to the water course, e.g. by river dams, watersides and harbor constructions [BOR 07], which influence quality, quantities and fate of contaminants associated with sediments as they are introduced to the sea. Dredged material can be disposed of at sea or ashore [BOL 06, SPE 08]. Sediments are an important source of different construction materials. The pollution of the marine coastal zone is affected by these factors. The natural dynamics, such as high energy meteorological phenomena (e.g. storms, intensive rains resulting in floods, high tides) determine further the fate of contaminated sediments in the marine environment [HEI 07].

Primary production in estuaries is often extremely high and many marine animals, such as fish, either pass the estuaries during their migration up the stream or spawn there, and sediments can play an important role in their life cycles. In polluted coastal ecosystems, biodiversity may decline, even of the unicellular organisms [CHE 09]. High nutrient and organic matter levels can intensify phytoplankton blooms resulting in eutrophication and associated with it high detritus concentration, hypoxia/anoxia, etc. [DUC 08]. The high concentration of plankton and detritus play an important role in transfer of contaminants to sediments and their accumulation therein [KON 01, KOW 03]. Moreover, intensive flocculation occurs in the mixing zone of freshwater that carries a lot of inorganic and organic colloids and seawater. The formed aggregates scavenge both the molecular and ionic contaminants from water column to sediments. Conversely, sediment bioturbation and microbial activity release contaminants to pore water and the superjacent column of water by resuspension and remineralization of sediments [DON 07].

These dynamics explain why estuaries, lagoons and other aquatic basins at the shoreline may serve as important buffers in the transport of contaminants to the sea by acting as natural traps and by providing

a variable level of natural biodegradation treatment of waste substances [EDL 06]. Contaminants sorbed by coastal sediments may be released either by desorption or resuspension of the fine sediment fraction, and their subsequent transfer at first to the water column, and distributed further along and off the shore with the local currents. Hence, coastal marine sediments can generally be considered as accumulation areas of contaminants, which can pose a real threat to the marine life and to the health of people (e.g. [HAR 06, ZON 07]).

From the above-mentioned elements, it becomes evident that chemical monitoring of coastal sediments is crucial. However, sediment monitoring of the coastal environment was not so readily mainstreamed in regular observation programs. Before the adoption of the WFD [EU 00], different marine conventions established the basis for contaminant monitoring of the marine environment, including the sediments in the coastal zone (see Chapter 1). This was not done in the same way in the different regions. When the WFD was introduced, “coastal monitoring” was also first understood as “surface water monitoring”. However, further work by experts, e.g. the SedNet project [SAL 04], resulted in further elements for the monitoring of priority hazardous substances addressed in the daughter directive 2008/105/EC (see Chapter 1). This directive requires monitoring of contaminants in sediments for temporal trend assessment. The Marine Strategy Framework Directive treats the marine waters as encompassing “waters, the seabed and subsoil”, not only as water [TUE 09]. Now that the legislative basis is completed, the development has begun to harmonize the various monitoring programs as much as possible, with the establishment of contaminant lists for monitoring, sampling and analytical procedures (including reference material preparation), as well as the development of new, more effective and less costly monitoring methods and techniques to be used in the coastal zone (e.g. [BOR 04, QUE 06, BAR 07]). It is worth underlining that such developments were not already very easy for seawater alone [BOR 05]. Despite these complexities, instead of discussing all aspects of sediment monitoring, this chapter highlights the role of sediments in coastal monitoring on the basis of four issues that are of high current operational or scientific interest.

3.4.2. Sediment monitoring in the WFD context

The WFD is described in Chapter 1 [EUR 00]; it represented a big step from the earlier sector approach to a more integrated approach of water management [CAR 07, QUE 07]. With the consensus within the scientific community about the important role that sediments play for the quality of the water systems as they can act as a source or as sink of contaminants, the regulatory framework included sediment matrices in the context of chemical status monitoring of marine coastal waters. Controversies and debates took place within the scientific community about the development of a methodology for including sediments in the definition of the chemical status concept [CRA 03, BOJ 04, KOW 11] until reaching the present situation regarding sediment monitoring and related EQS (see Chapter 1). Methods are developed to assess the chemical and ecological status of European waters in a pragmatic, holistic, rational and science-based approach, achieving a broad consensus between scientists, policy makers and stakeholders [BOR 05].

3.4.3. Chemical monitoring in estuaries for coastal management

As indicated in the introduction of this chapter, the sediment quality is a result of anthropogenic activities typically taking place in the surrounding areas and, often even more importantly, of socioeconomic activities and the biophysical conditions in the whole catchment [SAL 05]. Chemical monitoring of sediments has to be designed to provide the stakeholders with reliable data both on the status and functioning of the ecosystem and in support of such important uses such as management of navigation, tourism or fishing and agriculture. In estuaries with large tides, distinct contamination gradients are usually observed from the inner to the outer parts. Contaminant concentrations in coastal sediments are usually low, and, particularly for organic contaminants, the analytical uncertainty of measured concentrations is high. Temporal trends in the sediment quality may be detected only over a long time period. Important common features of estuaries determining their sediment dynamics are the tidal currents, the transition from freshwater to saline water and a

zone with high turbidity. However, they vary widely in terms of size, tidal range, freshwater inflow and fluvial sediment input. The estuarine particulate matter is subject to complex hydrodynamic processes. Once deposited, its reworking can take place almost immediately, or can last years, decades or even still much longer. Any monitoring program, and the assessment of contaminant monitoring, data has to take these specific features into consideration [JAY 97]. On the contrary, monitoring data can be valuable to better understand the complexity of transport, mixture, deposition and erosion of fine particulate matter and the associated contaminants in estuaries. An improved understanding of sediment transport in estuaries may support the optimization of sediment management, and it could render the assessment of the input of particle-bound contaminants to the marine environment more reliable.

3.4.3.1. Monitoring design for particle-bound contaminants in estuaries

As particle-bound contaminants strongly tend to accumulate in fine-grained particles and organic matter, a correction for differences in grain size distribution (normalization) should be performed prior to data assessment, unless samples have a comparable composition [ACK 83, KER 02]. OSPAR monitoring guidelines [OSP 02] recommend additional normalization with co-factors to compensate for further differences in sample composition (e.g. organic matter and clay content, Fe/Mn-oxide coatings).

The design of a program for monitoring contaminants in mobile sediments of the main body of estuaries should take into consideration: (i) the hydrodynamic conditions of the estuary, (ii) the expected variability of normalized contaminant concentrations, (iii) potential local sources of contamination and (iv) sedimentation rates.

For trend monitoring, recently deposited surface sediments, representing the time period under consideration, should be sampled, e.g. from areas with low energy and high sedimentation rates (e.g. [ORE 97]). In the main body of estuaries, the upper layers of sediments are likely to be well mixed to depths of up to 10–20 cm by continuous deposition and erosion processes, and thus represent the

current contamination status at the sampling site. Where areas of low energy are lacking, an appropriate alternative to sediment sampling may be the sampling of SPM. For the latter, preferably passive sampling devices, such as sedimentation basins, are used. SPM samples have the advantage over sediment samples to represent a defined time period of usually 1–4 weeks. Furthermore, in areas with low deposition rates, sampling of sediments might include contaminated deposits from the past or natural sediments with regional background concentrations.

At predominantly fluvially-dominated sampling sites near the entrance of an estuary or at mainly marine-influenced stations near the mouth of estuaries, contaminant concentrations in particulate matter are usually not affected significantly by changing freshwater discharge, and a sampling frequency of two to four times per year should be sufficient for detecting a temporal trend due to a change in contaminant input. However, at sampling sites near the river mouth, concentrations are often near marine levels and difficult to detect. At sampling sites in the mixing zone of marine and fluvial particulate matter where contaminant concentrations usually are subjected to significant variations depending on freshwater discharge, a higher frequency of 12 samples per year is strongly recommended. For the assessment of temporal trends due to changes in contamination input, either samples at similar freshwater inflow or annual mean values should be used. Sampling sites in the mixing zone of estuaries are of special interest, as monitoring data may be used to derive information on the dynamics of fine-grained particulate matter.

Sediment cores from depositional areas may be used for retrospective temporal trend monitoring by analyzing separate layers. However, the assessment should take into consideration diagenetic processes in deeper layers, which tend to be anoxic [FAR 91, GOB 97, KER 02]. Furthermore, contaminant loads retained in depositional areas may be estimated from analyses of sediment cores. Analyses of contaminant concentrations in surface layers in depositional areas may give indications of current deposition or erosion tendencies, e.g. following construction works in estuaries.

3.4.3.2. *Summary*

This chapter can be summarized as follows:

- monitoring provides a survey of temporal trends and spatial distribution of contaminants in fine-grained particulate matter in estuaries. These may be used, for example, to assess the effectiveness of measures to reduce contaminant input or to check compliance with the non-deterioration principle of the EQS Directive (see Chapter 1). Furthermore, potential local sources might be detected;

- contaminants may be used as tracers for the transport of fine-grained particulate matter and for validating the results of numerical modeling of sediment transport. An improved understanding of sediment transport processes and controlling factors in estuaries is of utmost importance for planning and optimizing a sustainable sediment management, e.g. of construction works or dredging and disposal activities. In estuaries, sediment management for navigation is of special interest, as tidal currents result in large amounts of sediments to be dredged for maintaining the navigation channels. Monitoring results in deposition areas as potential secondary sources of contamination should be considered, when measures for improving water bodies in the course of the implementation of the WFD are planned, in order to avoid a potential release of the contaminant burden of the past;

- information on the impact on sediment dynamics of management measures that had been carried out may be derived from changes in results from chemical monitoring.

Monitoring data may contribute to a better knowledge of the retention of contaminated particulate matter in estuaries, and to provide a more reliable assessment of particle-bound contaminant input to the sea. A reliable input assessment is required for the implementation of the MSFD.

Analytical Methods

4.1. Trace elements

4.1.1. Introduction

Larsen *et al.* [LAR 11] reviewed the status of analytical methods for minor trace elements, in particular heavy metals, a summary of which is given in this section. Contrarily to synthetic substances such as TBT, trace elements are naturally occurring substances and therefore a “background” concentration has to be considered, which influences the way data assessment should be treated. The environmental impact of trace elements varies with different abiotic factors, e.g. pH, alkalinity and salinity; in addition, many trace elements are essential elements, used in biomolecules, and as such actively taken up or kept out of the organism by exudates that form complexes, e.g. algae and copper [WHI 01, AND 10]. The origin and fate of trace elements are discussed in many textbooks, and regulatory aspects have been considered in Chapter 1, in particular substances listed by conventions and for which EQS have been set in the WFD framework.

Methods for trace element analysis can be performed in a number of independent ways, based on the electrochemical, absorption, emission, fluorescence or X-ray properties of the element in question. Examples of the current methods are given below, with a short

description of their benefits and shortcomings [LAR 11]. Historically, electrochemical methods have been used successfully for measuring amalgamating trace metals (Cd, Pb, Cu and Zn) in seawater using a mercury drop electrode, whereas atomic absorption spectroscopy in different forms was the method of choice for sediments and biota. In the 1990s, ICP-MS was introduced and is nowadays the most widely used instrument for general trace element analysis owing to its applicability to almost the whole periodic table. For special applications, especially in sediments and for screening, different methods employing X-ray remain useful [WES 08] and for very precise and SI-unit traceable results, neutron activation analysis (NAA) and isotope-dilution ICP-MS are currently available.

4.1.2. Digestion methods

Digestion techniques usually involve heating samples in acid. A short description of methods is summarized in the following; technical details are provided by Larsen *et al.* [LAR 11]. In the first place, heating solid samples and acid can be performed in a number of ways. The main distinction is between open and closed digestions.

In open digestion systems, temperature is limited to the boiling point of the acid mixture or acid/peroxide mixtures used, and combinations of perchloric and nitric acids were common in the 1970s. The main concern in using open digestion system is the possible loss of volatile elements, e.g. mercury, which is often minimized by a reflux system. In open vessels, heating can be achieved by hotplates, or specially designed blocks that fit the digestion vessels and heat the acid from all around.

In closed digestion systems, the sample and acid are heated in a closed vessel (inner Teflon cylinder), often called a bomb (which is an indication of what may happen when the pressure exceeds the strength of the container), and the released gasses increase the pressure inside the vessel, so temperatures above the normal boiling point of the mixture are achievable. For closed vessels of stainless steel, an

ordinary oven can be used, typically at 250–300°C for more than 4 hr and cooling overnight. Microwave ovens have become the standard heating source in most modern laboratories since their first introduction in the 1970s [ABU 75] because of a higher energy output, a better controlled distribution of microwaves inside the oven and the possibility to operate batches from up to 64 samples at a time.

The simplest digestion method is by nitric acid, which introduces a minimum of interferences in the subsequent analysis [LAR 11]. Oxidation can be boosted by addition of hydrogen peroxide, perchloric or hydrochloric acid for samples with high fat content (especially for biota samples), e.g. for metals such as Hg, Cu and Zn, or addition of hydrofluoric acid in combination with *aqua regia* in cases of siliceous minerals [LAM 98]. For metals that are predominantly present in organometallic form in tissues, e.g. As, Hg and Se, special care should be taken to break the organometal bond, especially if the analytical method is hydride generation (HG)- or cold vapor (CV)-AAS (see section 4.1.2.3) as these methods require a specific oxidation state of the analytes [LAR 11]. For problematic elements such as Al, Fe and Cr in samples with high silicate content (e.g. some plant materials, suspended matters or sediment samples), hydrofluoric acid may be the only mineralization solution (with all the care required considering its potential health hazard), followed by evaporation to dryness and redissolution in nitric acid after digestion or neutralization with boric acid. This step leads to a complex sample matrix that can result in high blanks and detection limits, and standard addition or matrix-matched calibration should then be used in the final analysis [LAR 11]. Digestion may be based on mixtures of different acids, e.g. nitric and hydrochloric acids (*aqua regia*) after grinding to a fine powder [DJI 03]. In all cases, digestion methods require a careful optimization and checks of analyte recovery, e.g., through testing on certified reference material that matches analyzed samples [LAR 11]. For sediment analyses, it is usually necessary to use hydrofluoric acid for total metal determinations, dissolving the silica in sand particles. Many methods use strong mineral acids (nitric acid or *aqua regia*) instead. All methods are generally prescribed in

geological surveys, or if comparison to non-invasive elemental analysis (X-Ray, INAA) is required. Strong acid digestion methods are more relevant for environmental studies, as metals in the sand fraction are released on a geological timescale, whereas clay--silt (<63 μm fraction) bound metals can be ingested and released in sediment living organisms' digestion tract.

4.1.3. Preconcentration methods for seawater analysis

Analysis of trace metals in seawater is challenging due to extremely low concentrations of metals in seawater and the considerable influence of matrix elements such as Na, Mg, Ca, K and Cl [LAR 11], hence preconcentration is necessary, especially for ICP techniques (see section 4.1.2.4) for which seawater causes signal suppression due to the influence of easily ionized matrix elements on the plasma (Na and K in particular) as well as signal drift caused by accumulation of salts on the cones and lenses of the ICP-MS. Simply diluting the seawater with 18 M Ω water is a way of reducing the matrix effects that can be applied in polluted coastal waters, but most often it results in inadequate instrument sensitivity or high blank levels due to the very low metal concentrations in seawater, especially in open-ocean samples. A common way of solving the analytical problem of seawater analysis is by preconcentrating the metals. A column filled with a chelating resin (see examples in Beck *et al.* [BEC 02] and Larsen *et al.* [LAR 11]) followed by a rinse of matrix elements from the resin and elution of the metals allows one-step concentration and simplification of the matrix [BEC 02, EVA 05]. Preconcentration and elution can either be made "off-line" with subsequent analysis of the collected eluate [FER 02] or "on-line" as part of the (ICP-MS) workflow [BEC 02]. Using the on-line method gives an elution profile (concentrations vs. time) of the metals and the average peak or integrated intensity or the maximum peak intensity is used as proportional to the sample metal concentration. Performing the preconcentration step on-line greatly reduces the contamination problems associated with the off-line method. Special precautions may have to be applied when working with trace metals in seawater as

contamination problems are notoriously difficult to overcome, particularly with more common metals such as Zn and Ni. This means that all equipment in contact with the water (bottles, filters, vials, pipette tips, etc.) have to be thoroughly acid-cleaned prior to use and the handling of samples, etc., should take place only in clean room or clean bench facilities.

Liquid–liquid extraction (LLE) can be performed for divalent metals by complexation with ammonium pyrrolidine dithiocarbamate (APDC) or diethylammonium diethyldithiocarbamate (DDDC) followed by extraction with solvents such as methyl isobutylketone (MIBK), Freon or chloroform. The organic phase is then separated, and either evaporated and the metals are redissolved, or the complexation is broken by nitric acid and back-extracted to water phase [GRA 99, KOM 06]. Preconcentration of a factor of 40–400 is achievable with 400 mL sample to 10 mL final volume for flame/ICP or 1–2 mL final volume for graphite furnace analysis. All solvents and glassware need to be scrupulous cleaned before use to avoid contamination. The method is applicable to Cd, Co, Cu, Fe, Ni, Pb and Zn. This being said, a lot of variations on the original method exist, and other complexation agents can be used for extending the range of analytes. Comparisons of different solvent extraction versus SPE and coprecipitation have shown that a good agreement is found between different methods [KOM 06]. Possibilities for automation and the achievable detection limits for the available methods are therefore the main selection criteria, together with environmental safety (e.g. restrictions in the use of Freon). To avoid the large amounts of solvents usually employed in LLEs, *microliquid extractions* have been employed for a number of species. A review of the techniques by Dadfarnia and Shabani [DAD 10] highlights their potential with enrichment factors of 20–560 for metals and up to 1,000–5,000 for organotin compounds and organomercury.

4.1.4. Atomic absorption and emission techniques

Both AAS and atomic emission spectrometry (AES) techniques rely on the dissolution of samples into a clear liquid that can be

aspirated or pipetted into the analytical detector system. This step represents a potential source of error since digestion systems are not exempt from possibility of losses, contamination or improper dissolution of the matrix before the analysis [LAR 11]. Current AAS developments now focus on preconcentration and speciation techniques for routine applications [EVA 05]. The first AAS and AES techniques used flames or (graphite) furnaces to ensure that the elements were atomized, but in the last decade, ICP became the preferred way of ensuring atomization, either measuring atomic emission from the plasma or extraction of ions by a mass spectrometric detector.

4.1.4.1. Atomic absorption and fluorescence spectrometric techniques

4.1.4.1.1. Flame AAS

Larsen *et al.* [LAR 11] report that AAS has been the cornerstone of elemental analysis from the beginning of the 1980s until the early 2000s. Although the technique is now surpassed in many ways by ICP-MS (see below), the detection limits and precision of AAS methods can still offer good precision and reproducibility of less than 1–2% over several hours of operation for single element studies. Operational details about atomization are given by Larsen *et al.* [LAR 01] and will not be repeated here. Matrix-matched standard curves or standard addition calibration is the usual way to counter possible matrix effects. The flame temperature (1,700–3,150°C), either air–acetylene or acetylene–nitrous oxide, is enough to atomize most metals, but the sensitivity is not always sufficient for measurements of environmentally relevant concentrations, except for minor constituents and the more low melting metals such as Zn, Cu, Al and the alkali metals.

4.1.4.1.2. Graphite furnace AAS

Flameless atomization systems using a graphite furnace [LVO 90] have enabled to reduce the sample amount and to increase sensitivity, collecting sample (5–100 µL droplets) on a small platform,

which helps preventing condensation of the sample. A stepwise heating is undertaken, allowing to remove water by drying (80–150°C) and organic matters by ashing (400–700°C) to avoid interference in the atomization step, preatomization (1,000–1,500°C) for removing potential interfering metals of lower volatility and finally atomization and measurement (2,000–3,000°C). The technique is especially suitable for Ag, Cd, Cr, Cu, Ni and Pb, whereas the sensitivity for Zn is too high, rendering blanks prohibitively high unless clean room conditions are used [LAR 11]. Commercially available graphite furnace AAS models allow simultaneous measurement of several elements by using multielement hollow cathode lamps or electrodeless discharge lamps, at the price of higher detection limits and less optimized temperature programs. Background correction methods (Zeeman effect, deuterium lamp) are used to cope with non-specific absorption, caused either by non-dissociated molecules in the light path, or scattering caused by particles like salt. Standard addition calibration is often used, allowing to cope with matrix effects. Albeit time-consuming, this calibration method is usually very accurate. With unknown samples close to the detection limit, the error is often underestimated. Any background signal independent of standard concentration is not corrected for using standard additions, so a separate determination of background in reagents and sample matrix must be subtracted after the standard addition. GFAAS is mainly applied to solutions. Good results are, however, obtained with solids using stirred slurry sampling for direct solid sampling [CAL 02]. The reproducibility of the results can be excellent if the slurry is uniform at the applied sampling volume [MIL 06].

4.1.4.1.3. CV and HG AAS

For volatile elements, e.g. hydride-forming elements As, Bi, Ge, Sb, Se and Te, the HG system offers an excellent sensitivity. All these elements (As(III), As(V), Bi(III), Ge(IV), Sb(III), Sb(V), Se(IV) and Te(IV)) form volatile hydrides when reduced by atomic hydrogen (generated by addition of NaBH_4 to acidified solutions) and are subsequently separated by an inert gas stream, usually Ar, which leads them toward an electrically heated quartz tube where atomization is

performed at circa 1,000°C. Detection limits for these elements are typically reduced with a factor 5–25 compared to direct AAS (typically 0.1–0.2 µg/L), and additionally, matrix effects are greatly eliminated as only matrix carried to the quartz cell by the Ar stream is seen by the detector. For the determination of total element contents, different oxidation states have to be converted, e.g. Se by treatment with strong HCl at elevated temperature to ensure oxidation of Se(II) species to the hydride-generating Se(IV).

The determination of mercury via CV-AAS involves an analogous mechanism, making use of the easy reduction (using Sn^{2+} as a reducing agent) of both Hg^{2+} and Hg_2^{2+} to metallic Hg in both acid and alkaline mediums. The formed Hg(0) is removed via a stream of Ar bubbles and leads toward a pyrex cell. Since the vapor contains Hg in atomic form, heating is redundant (hence the name CV), except to avoid water vapor in the cell, and the absorbance can be readily determined. Sensitivity goes well beyond the µg/L level, a must for environmental analysis of this very toxic element. For biota, NaBH_4 is used often, while sediments and water samples normally are reduced with Sn^{2+} . To increase sensitivity, gold traps can be used, forming Hg-Ag amalgam, which can be thermally desorbed above 450°C. Detection limits are typically in the 1–10 ng/L range with amalgamation, depending on the time/volume of sample preconcentrated onto the gold trap [LAR 11].

4.1.4.1.4. Atomic fluorescence techniques

Atomic fluorescence techniques are usually based on flow injection or a similar system for online preparation, typically hydride or CV generation and a gas/liquid separation. The main advantage of fluorescence compared to atomic absorption techniques is the sensitivity, typically a factor 10–100 better than AAS and the even higher specificity as excitation and fluorescence signal must both match the measured element. AFS systems for mercury are commercially available for analysis of gasses, liquids or solids [LAR 11]. Hydride-forming elements can be analyzed by atomic fluorescence methods as described under HG AAS, except the quartz

cell can be exchanged for a hydrogen flame. Detection limits are typically better than AAS methods.

4.1.4.2. *ICP spectrometric techniques*

4.1.4.2.1. ICP-AES

The ICP is used as a source of atomizer for atomic emission spectrometer (Atomic Emission Spectrometry (AES) also called OES for optical emission spectrometry) or ions into a mass spectrometer system (using the same sample introduction system and excitation/ionization source). The liquid sample is nebulized using a mechanism comparable to flamespectroscopy. The sample is introduced in the plasma at the end of a torch via a carrier gas. The plasma torch can be placed either horizontally (especially suited for AES) or vertically. The temperature is generally 6,000–10,000°C, but can be even higher. The plasma is a complex mixture of molecules, ions, atoms and electrons. Excitation and ionization occur through collision of particles [LAR 11]. Due to collisions, new charged particles are formed constantly, temperature increases and the plasma is kept alive. The stability of an ICP is outstanding; the noise is of the order 0.1%. Originally, the detection systems consisted of a polychromator and a number of photomultiplier tubes to be set at the selected first-order wavelength. Nowadays, the signal from the plasma is split up, recombined and read out by a chip, allowing analysis of a virtually unlimited amount of elements in the same run [SWE 89]. Spectral overlap is usually avoided by the automatic selection of specific wavelength for a certain analysis type (with its specific interferences). Another possibility is to use several wavelengths to make more robust calibrations [SCH 09] or modeling of interferences and automated subtraction.

4.1.4.2.2. ICP-MS

The ICP provides an excellent ion source for introduction into a quadrupole MS. More than 90% of metals are present as monovalent ions M^+ at typical plasma temperatures. The mass spectrum consists of a number of ions, i.e. monovalent metal ions (with masses related to the abundance of isotopes), poly-(usually di-)valent ions, molecular

ions of metal hydrides, oxides or hydroxides and molecular ions of plasma gas and solvent constituents [LAR 11]. For most trace metals, mass interferences can be overcome by appropriate choice of the isotope and acid mixture; other approaches, e.g. mass-correction, are also used [MAY 98]. Tuning of the ICP-MS is required to minimize interferences, i.e. oxide and double-charge formation. A number of different MS-detectors have been used with different mass resolution R , defined as the mass divided by the mass difference of 10% peak overlap for equal height peaks. The quadrupole has an R of 10, time-of-flight (TOF) detectors have an R of 500 and high-resolution (HR) sector field MS has $R = 4,000\text{--}10,000$ mass unit resolutions, capable of isolating even ^{75}As from ArCl interference. Moreover, the isotope dilution ICP-MS method is a very precise and accurate (albeit expensive!) technique, involving the addition of a small amount of a certain isotope of the analyte to the sample, which behaves in chemically identical way. This means measuring the isotope along another isotope at the same time, and knowing the normal isotopic distribution of the analyte, the original concentration in the sample (the sample “dilutes” the spike) can be calculated by the ratio of the added isotope to the natural isotope(s). When using isotope dilution, mass interferences need to be carefully controlled, since they can seriously affect the measurement. The combination with HRMS leads to extraordinary good results [LAR 11].

HR ICP-MS can be used for increased sensitivity (down to pg/L) and overlapping peaks [ZHE 03], which for many nuclides is needed to be able to measure them at natural levels. In this context, the challenge is keeping samples from contaminating sources, so extreme caution and ultra-clean environment and reagents are needed. For seawater, samples can be analyzed by dilution using the purest reagents, while for sediments, the usual problem is dissolving the matrix before introduction into the plasma (see above), but dilution and the higher peak resolution can solve most of the remaining matrix problems. HR-ICP-MS instruments are still used mainly for research, but improvements in the software for tuning and stability are increasing the applicability in the environmental laboratory, although costs remain high [LAR 11].

4.1.5. (Instrumental) neutron activation analysis

NAA is a sensitive non-destructive analytical technique that has been in use since the early 1960s [FUK 59] for multielemental analysis of major, minor and trace elements in samples from almost every field of interest or for any matrix (from water samples, to any organic matrix, to sludge or sediment or solid samples). This technique allows discrete sampling of elements as it disregards the chemical form of a sample, and focuses solely on its nucleus. Several applications are well known in marine environment, e.g. major inorganic constituents and rare earth elements in marine sediments, trace element analysis in marine organisms such as fish and shellfish, elemental composition in several marine bioindicators (e.g. for pollution) or as a tool in the determination of extractable organohalogens in organisms from the marine ecosystem. Although the technique was mainly used as high-precision research tool for academia, the focus has shifted nowadays to a reference technique recognized by the International Bureau of Weights and Measures (www.bipm.org) not only as a primary method for validation of some chemical techniques, including the certification of reference materials for elemental analysis (see Chapter 2), but also as a well-established commercial analysis for multielemental analysis of mainly trace elements with high precision. NAA is very sensitive for the determination of halogens that are difficult to be determined by other techniques: Fluorine (using a fast pneumatic system down to 2 mg/kg), chlorine (mg/kg level), bromine ($\mu\text{g/kg}$ level), iodine (50 $\mu\text{g/kg}$ level using ENAA), volatile elements (e.g. arsenic, selenium, mercury) or elements that are difficult to dissolve (e.g. chromium and tin). When used in combination with appropriate separation techniques, NAA can also provide valuable information about trace element speciation, e.g. for As, Se, Cr and Sb [SAL 92].

In NAA, the sample is bombarded with neutrons, causing the elements to form radioactive isotopes [GLA 10] and the analysis is performed using γ -ray spectrometry on dried or freeze-dried (homogenized) samples that are packed in irradiation vials (typically polyethylene) together with flux monitors and quality control

material – no additional sample preparation (extraction, dissolving or dilution) is needed [LAR 11]. Once packed, samples are placed near the core of a nuclear research reactor and irradiated for a predetermined time span, so that the neutrons emitted by the reactor interact with the nuclei rendering them radioactive. The compound nuclei relax to more stable configurations through the emission of nuclide-specific γ -rays, unique in energy and half-life time $t_{1/2}$ (varying between seconds and several years). This emitted radiation is a fingerprint and allows for any identification of the targeted element(s) in the sample, and the radiation intensity allows quantification. NAA is capable of providing a comprehensive determination of the samples' total elemental content regardless of oxidation state, chemical form or physical state of the desired element(s), so all sources of systematic or random uncertainty are well controlled. The method is non-destructive and the sample can be re-analyzed, if necessary. In most cases, the radioactivity level after 1-year storage is sufficiently low to return the sample to the customer. NAA laboratories are still active in several European countries (see [LAR 11]). However, the cost of irradiations in the nuclear reactor and the cost of the computation make this technique relatively expensive for one element compared to most chemical techniques. On the other hand, the very high accuracy of the method and the limited need for sample pretreatment, minimizing recovery problems, loss of volatile components or sample contamination, need to be stressed.

4.1.6. X-ray techniques

X-ray techniques are often used routinely in determining the extent of soil pollution. In marine analyses, these techniques cannot be used directly for sediments owing to their water content; although the methods are non-invasive, they often require some pretreatment like drying and tablet pressing before use. The major elements (Ca, Cu, Fe, K, Rb, Sr and Zn) can often be determined with X-ray methods [WES 08]. Detection limits and precision for use on sites are not as good as for standard analysis for portable instruments [MEL 04], but the methods can be used for characterization purposes and semi-quantitative analyses for determining the extent of pollution at contaminated sites. Methods based on proton-induced X-ray emission

(PIXE) have been used for sediment analyses, and water analysis after freeze drying of the water, or dried filter samples. PIXE can be applied to very small samples with minimum sample preparation. The combination of electron microscopy and X-ray methods makes it possible to scan sediment cores or look on single particles in inhomogeneous samples [LAR 11].

4.1.7. Electrochemical techniques

Electrochemical methods may be used for various elements and samples, but their real strength lies with seawater analysis [LAR 11]. The method is cheap (the equipment cost is an order of magnitude lower than for AAS and ICP), but it requires skilled operators and is time-consuming. It is a multielement method, as up to five elements can be determined in the same run. The equipment used for it is light and can easily be brought onboard a research vessel. Miniaturization allows nano-electrodes to be deployed in otherwise inaccessible places. Nearly all electrochemical methods use mercury, so strict procedures for collection and reuse of the mercury are imperative. Also, environmental mercury analysis requires a room separated from the one used for electrochemical analysis.

4.1.7.1. Polarography

Polarography was invented by Jaroslav Heyrovsky [HEY 66]. In this technique, a dropping mercury electrode is immersed in the sample, the voltage of the electrode is changed linearly and the current through the mercury is measured. There is a sudden current change at a potential characteristic for each element; this step is proportional to the concentration of that element.

4.1.7.2. Anodic stripping voltammetry

In the anodic stripping voltammetry (ASV) technique, three electrodes are immersed in the sample that has been adjusted to a specific pH: (1) The working electrode concentrates the elements in the sample, (2) an auxiliary electrode supplies current through the sample and (3) a reference electrode measures the potential of the working electrode. The reference electrode can be silver/silver

chloride and the auxiliary electrode can be anything, but is usually either carbon or platinum to prevent any interferences. The working electrode is the critical part of the method. In the first use of ASV, a hanging mercury drop electrode was used. Later, the mercury film electrodes were developed and the rotating mercury film electrode was the most sensitive electrode. The mercury film is electrochemically plated on either graphite or glassy carbon, the latter seems to be the preferential choice [LAR 11]. The analyses start with a concentration step, during which dissolved elements are electro-plated on the working electrode and amalgamated with the mercury film. The longer the plating time, the lower the detection limit of the method. During the stripping step, the potential of the working electrode is gradually increased and the electric current associated with the dissolution of the individual elements is recorded. Refinements as differential pulse or square wave measuring techniques can improve the accuracy and detection limit of the method. The method is perfect for measuring cadmium and lead in seawater, where detection limits of a few ng/L can easily be achieved. It is also very good for zinc, copper and bismuth. Details about ASV theory are thoroughly described, e.g., by Protti [PRO 01].

4.1.7.3. Cathodic stripping voltammetry (CSV)

In CSV, the equipment is the same as in ASV but the concentration step is performed at a more positive potential, e.g. at 0 V, at which several organic metal compounds are adsorbed on the mercury film. In the stripping step, the voltage is lowered gradually and the current associated with the reduction of the metal is recorded. The method needs an organic complexation agent for the metal to be analyzed (dimethylglyoxime for Ni and Co). CSV can be used for many elements, notably Fe, U, V, Cu, Zn, Mo, Ni and Co [VAN 86].

4.1.8. Conclusions

The method of choice for trace element determinations in modern laboratories today is ICP-MS, due to its versatility and low detection limits [LAR 11]. When only a few elements or especially Se and As (the elements most prone to mass-interference in ICP-MS), other

techniques like atomic fluorescence or atomic absorption can be a more cost-effective solution. For seawater samples, preconcentration ICP-MS or electrochemical methods are the preferred methods, whereas for fast screening of sediments, X-ray techniques can have advantages. The only methods currently available with direct traceability to SI units are isotope-dilution MS and neutron activation (see Chapter 5), with the possibility of high-precision analysis. For most applications though, use of LRM or CRMs is sufficient to ensure the quality of the analysis.

4.2. Chemical species

4.2.1. Introduction

Analytical techniques for speciation analysis have been reviewed in depth by Amouroux *et al.* [AMO 11]. This section provides a synthetic summary of the main features relevant to marine metrology. When referring to “speciation”, definitions have been discussed for long as this includes a wide variety of analyses, ranging from the determination of well-defined “species”, e.g. oxidation states of elements or organometallic compounds to forms of elements that are operationally defined (i.e. related to an extraction procedure). Details about the different categories are discussed in the literature [QUE 98b].

In the marine environment, metallic species are present in a variety of chemical formulations such as free ions, inorganic complexes, organometallic species or large organic complexes, and the fate between different compartments of the environment and their impact on biological organisms are regulated mainly by their chemical forms [AMO 11]. Biological organisms are able to accumulate within cells (bioaccumulation), to transform chemically (biotransformation) or to neutralize by specific ligands (biocomplexes) forms of more or less toxic metals and metalloids. The mobility, reactivity and toxicity of metals and metalloids are also explicitly dependent on their molecular forms (inorganic, organometallic, etc.). Understanding biogeochemical cycles and processes of contamination of metals and

metalloids is hence directly related to the knowledge of physicochemical forms in which the element is engaged and its interaction with the environment, and this is strongly dependent upon measurement capacities. However, methodological limitations are often encountered due to the presence of these compounds at trace levels in marine environmental compartments (ng/kg to $\mu\text{g/kg}$). The methods developed to study the fate of these traces chemical forms of metals and metalloids have been traditionally based on specific and sensitive “atomic” detection techniques, thus severely limiting the molecular information available [AMO 11]. This limitation is nowadays solved by the development of hyphenated methods coupling separation techniques and specific techniques for sensitive and selective detection by atomic spectrometry (optical, mass) (Figure 4.1). Current techniques involve a combination of sensitive analytical techniques, in particular chromatographic separation (GC or HPLC) coupled on-line with a multielement detection system, e.g. ICP-MS.

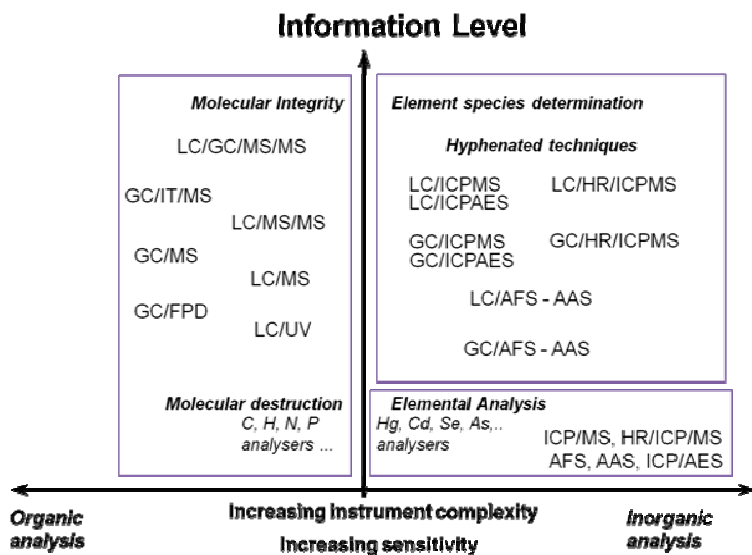


Figure 4.1. Complementarities and available techniques for element species determination by hyphenated methods: a gap between organic and inorganic analysis

4.2.2. Labile/complexed fractionation of metal species

4.2.2.1. Seawater analyses

The determination of metal species in seawater is mainly driven by studies about their liability and mobility in the marine environment, which determines their availability (and toxicity) to the biota. Several studies have demonstrated that the uptake is only related to the free ion activity of the metal or to the labile metal forms well than to the total concentration [LOR 02, MEY 04]. Although some attempts have been made to determine free metal concentrations, available analytical methodology is usually capable to determine a group of species better than a unique form [AMO 11]. The most general division includes free metal and inorganic complexes as labile fraction and organic complexes, usually more stable, as non-labile fraction. This classification is however operationally defined, hence strongly dependent on the analytical technique used. This is a major flaw owing to the variety of techniques developed for dissolved metals, requiring in-depth checking of a correct assessment of data. An exhaustive revision of these techniques and their applications to metal speciation in environmental waters can be found in the literature [BUF 05, PES 09, LÓP 10]. Examples and recent applications in the marine environment are given by Amouroux *et al.* [AMO 11].

As mentioned in section 4.1.7, ASV may be used to determine the electroactive fraction of metal (ASV-labile); the metal fraction associated with organic ligands may also be determined with an additional measurement of total metal concentration. In addition, ligands concentrations and the related stability constants can be calculated. ASV has been successfully applied to differentiate the species of lead, copper, cadmium and zinc in different marine and estuarine areas [CAP 90, SCA 95, GER 96, AND 06, JON 07, BRA 07]. CSV or cathodic stripping chronopotentiometry has also been used in speciation studies in seawater samples [VAN 91, ELL 01, MAR 04, BRA 07]. A common advantage of the electrochemical techniques is their capability to perform *in situ*

speciation measurements [BUF 05]. Although electroanalytical techniques offer a very interesting alternative to speciation studies in seawater, a main drawback is that only a few elements can be determined with these techniques (mainly copper, cadmium, lead and zinc). For this reason, alternative non-electroanalytical devices based on ion exchange, DGT gels or permeation liquid membranes (PLM) may be used (see Chapter 3), e.g. DGT has been successfully applied to *in situ* metal speciation in freshwater and seawater since its development in the mid-1990s [DAV 94, ZHA 95]. Labile species (free ion, inorganic and also some labile organic species) may be determined after *in situ* preconcentration in an ion-exchange resin and elution by nitric acid before analysis with ICP-MS. A direct analysis can also be performed by X-ray fluorescence [AMO 11]. PLM have shown their applicability to determine free metal concentration as well as organic and inorganic metal complexes in natural waters [LÓP 10]. Nevertheless, the methodology has been developed mainly to separate labile and non-labile fractions of copper and, to a lesser extent, lead and cadmium [SLA 04, PAR 04, ROM 05, BAY 06, GUN 08]. Some comparison has been made between this methodology and DGT or voltammetry in estuarine and seawater samples [NDU 05, SLA 09].

Finally, ion-exchange techniques are commonly used for seawater analyses owing to their simplicity, easy automatization, multielemental analysis, simultaneous separation and preconcentration and the possibility of *in situ* speciation. Techniques are based on the adsorption of free ions and some inorganic complexes onto a resin (e.g. Chelex-100, C₁₈, etc.) and their later elution with an appropriate solvent. Depending on the resin characteristics, the species retained are different and speciation can be operationally performed. Some methods use several columns, which allows the separation of several fractions [GRO 94, ABO 00, ABA 03, ALB 08]. In spite of the great versatility of these systems because of the variety of sorbents available, speciation is strongly dependent on the type of resins used, which may affect the comparability of results of monitoring in different areas. The possibility of automatization of these systems

combined to *in situ* sampling, and even speciation, makes them as an attractive alternative for the classical marine monitoring of metals [POI 07].

4.2.2.2. Sediment analyses

Sediments play an important role as a sink of metals in marine environments, but they can also act as a source of these elements depending upon the physicochemical conditions. The speciation of metals in sediments is closely related to metal extraction capacities under specific environmental conditions, i.e. sediments can be divided into operationally defined fractions that can be selectively extracted using the appropriate reagent. A popular method is sequential extraction, which allows the determination of metals contents associated with different sediment phases. In these procedures, the residual phase from successive simple extractions is used in the next extraction step following a sequence until the last fraction (residual) is extracted. Due to the huge bibliography available about sequential extraction, this text only includes the more relevant developments and recent applications on marine environment. For an in-depth review, the reader is invited to consult papers by Filgueiras *et al.* [FIL 02], Quevauviller [QUE 02b], Bacon and Davidson [BAC 08] and Rao *et al.* [RAO 08].

As previously stated, sequential extraction procedures are interesting to evaluate the mobility of metals under different environmental conditions. In this sense, the sequential extraction scheme proposed in the late 1970s has initiated a wide range of research on trace metals fractionation in solid samples [TES 79]. The method was first applied to the partitioning of trace metals (Cd, Co, Cu, Ni, Pb, Zn, Fe and Mn) into five fractions: exchangeable, bound to carbonates, bound to Fe-Mn oxides, bound to organic matter (including sulfide forms) and residual. The exchangeable fraction corresponds to the more labile fraction adsorbed on sediments, which could be released by ion-exchange or desorption processes and the fraction bound to carbonates would be susceptible to changes of pH. The metals that could be released under more reducing or oxidative

conditions represent the fraction bound to Fe-Mn oxides and those bound to organic matter, respectively. Finally, the residual fraction arises for metals from the mineral structures. Modifications of this scheme have been frequently undertaken to improve the fractionation of metals differentiating metals bound to the organic matter or trapped in the sulfidic fraction [CAM 95] as well as between the easily reducible fraction and the moderately reducible fraction [KER 86]. In addition to modifications in the extraction sequences and in operational conditions, changes in the extraction of residual fraction are usual in order to compare the results with those obtained from a total extraction procedure. In these cases, the same extraction methodology is used in both extraction of residual fraction and total extraction. Many comparisons between the different procedures have showed some unsolved problems associated with the sequential extractions, as lack of specificity of reagents, redistribution of analytes among phases, incomplete extractions and precipitation of new mineral phases or the dependence on sample pretreatment [USE 98, FIL 02, JOK 05, BAC 08, NEM 09].

The various methodologies (and the frequent modifications) make it difficult to compare data and develop quality control tools (see Chapter 2). Hence, for the sake of harmonization, the European Commission proposed a three-step sequential extraction methodology, which was later reevaluated and revised, to harmonize the available procedures [URE 93, RAU 99, QUE 02b]. The BCR methodology defines three fractions: acid soluble (exchangeable and bound to carbonates), reducible (bound to Fe/Mn oxides) and oxidizable (bound to organic matter and sulfides). Speciation studies carried out by this method usually include a fourth step to extract residual fraction [YUA 04, CUO 06, GAO 08]. In addition, certified reference materials have been prepared on the basis of the BCR methodology, thus improving the quality control in sequential extraction [LÓP 98, PUE 01]. This is the main reason for a preferred use of the BCR extraction procedure in the last years [QUE 02b]. Some of the most recent applications are shown in Table 4.1.

<i>Sample preparation</i>	<i>Separative method</i>	<i>Detection</i>	<i>Elements</i>	<i>Fraction</i>	<i>LOD</i>	<i>References</i>
Air drying and grinding/wet and not grinding	Three-step sequential extraction	ICP-MS	Ba, Cd, Co, Cu, Mn, Mo, Ni, Pb, Sc, Sr, U and Zn	Acid-soluble, reducible, oxidizable and residual	—	[GAO 08]
Sieving (63 µm)	BCR sequential extraction	ICP-MS	V, Cr, Mn, Co, Ni, Cu, Zn, Mo, Cd, Sn and Pb	Acid-soluble, reducible, oxidizable and residual	—	[YUA 04]
Air drying, grinding and sieving (63 µm)	BCR sequential extraction	ETAAS	Cd, Cr, Cu, Ni, Pb and Zn	Acid-soluble, reducible, oxidizable and residual	—	[CUO 06]
Air drying and sieving (1 mm)	Four-step and three-step sequential extraction	FAAS	Zn, Fe, Cu, Mn, Pb, Cr, Cd and Ca	Exchangeable, reducible, oxidizable and residual	—	[NEM 09]
Filtration (0.4 µm), filters washed with HCl and air-dried	Three-step sequential extraction	ETAAS/ ICP-AES	Cd, Cu, Fe, Mn and Pb	Acid-soluble, reducible and oxidizable	0.01 µg/g	[MAG 05]
Filtration (0.45 µm) and filters oven dried	Three-step sequential extraction	PSA	Cd	Weakly adsorbed metals, iron-manganese oxides, bound metals and organic/sulfide-bound metals	—	[WAE 05]
Filtration (0.45 µm) and filters oven dried	Three-step sequential extraction	CCSA	Cu	Weakly adsorbed metals, iron-manganese oxides, bound metals and organic/sulfide-bound metals	—	[WAE 05]

Table 4.1. Methods used for metal fractionation in marine sediments

Finally, sequential extraction procedures have also been applied to suspended solid matter, although only a few examples in marine (in particular estuarine) environment can be found in the literature [MAG 05, WAE 05] where interaction between particulate and dissolved fractions has important effects on the transport and availability of metals.

4.2.2.3. Marine biota analyses

Marine biota speciation analyses of metals are mostly related to the determination of biogenic organometallic complexes and compounds synthesized under high metal concentrations exposure and/or uptake, such as metallothioneins (MTs) and metallothionein-like proteins (MTLPs). The development of hyphenated techniques combining a separation technique as chromatography with a sensitive and element-specific detector as MS has enabled the characterization and quantification of metallothionein forms [SZP 00, PRA 02], examples of applications of which are given in [AMO 11]. The use of hyphenated techniques allows a better speciation of metal binding to different cytosolic ligands. A typical approach is based on the separation depending on the molecular weight, although applications to marine vertebrates are very limited [AMO 11].

4.2.3. Inorganic chromium species

4.2.3.1. Introduction

Several chemical forms of chromium exist but only trivalent chromium and hexavalent chromium are stable enough to occur in the environment [PET 05]. Cr(III) is considered as an essential micronutrient for humans and animals; in contrast, Cr(VI) is much more toxic than Cr(III) for both acute and chronic exposures. The toxicological disparity between Cr(III) and Cr(VI) is closely related to the chemical characteristics of each one, which also conditions the stability, mobility and bioavailability of these species in the marine environment [PET 05]. Analytical techniques generally used for Cr speciation in marine samples can be separated into two groups:

(1) methods that can determine Cr(VI) or Cr(III) after a pretreatment of the sample and (2) on-line hyphenated techniques allowing the determination of both species simultaneously. When applied to solid samples, most of these methods require a previous extraction step [AMO 11].

Spectrophotometric and colorimetric methods have been recommended for the determination of Cr(VI), e.g. the 16. EPA Method 7196 [USE 95b] and Method 7199 [USE 95] involving ion chromatographic separation. Electroanalytical methods are also used for the direct determination of Cr(VI). The most common method is stripping voltammetry that allows reaching ng/L levels [GRA 08]. Atomic spectrometric techniques (see section 4.1) such as flame atomic absorption spectrometry (FAAS), electrothermal atomic absorption spectrometry (ETAAS) and ICP-AES have been used for chromium speciation after a separation or isolation technique that provides selectivity for one species relative to the other. For samples with low levels of chromium, ETAAS and ICP-AES are more suitable than FAAS. On-line separation and detection methods have been developed in order to minimize contamination and losses of Cr species or redox conversion [COR 03]. From mid-2000s onward, ICP-MS became the preferred detection technique for chromium owing to its high sensitivity and selectivity. Coupled to ICP-MS, ion chromatography (IC) is the most widely used separation method. Several approaches have been used to achieve suitable chromatographic separation of chromium species. Anion-exchange columns [BAR 97, TIR 03, RAH 05] and columns with both anion- and cation-exchange capabilities [VAS 00, SÉB 03] have been explored. HR ICP-MS efficiently removes most interferences but its high cost is hardly affordable for routine laboratories.

4.2.3.2. Seawater analyses

Total chromium concentration in ocean waters is mainly in the range of 50–500 ng/L [SIR 00] and the dominating Cr oxidation state is found to be Cr(VI). Before Cr speciation analysis, it is recommended to simply filter seawater samples and store them

at -18°C to avoid Cr redox changes [SIR 97]. Acidification has to be avoided because Cr(VI) is immediately reduced into Cr(III). Different methods have been developed for speciation analyses of chromium in seawater and details are given by Amouroux *et al.* [AMO 11]. Only those allowing to reach concentration levels usually found in seawater are discussed.

Sample pretreatment techniques including separation and/or preconcentration are required in order to determine the low levels of individual Cr species even with the most sensitive techniques. The disadvantage of many of these methods is that one of the species is determined as the difference between total chromium (often obtained after reduction or oxidation) and the other chemical form of the element. Coprecipitation techniques have been used for the isolation of Cr(III) and Cr(VI) [CRA 80, AHE 85, PET 97, CON 06]. The concentration of Cr(VI) is obtained after coprecipitation of both Cr(III) and Cr(VI) onto iron(II) oxides and calculation of the difference between this fraction and the Cr(III) concentration. Coprecipitation is generally followed by determination of total chromium with AAS methods. Detection limits obtained are the lowest for Cr speciation in seawater samples because of preconcentration during the coprecipitation step and the removal of the saline matrix. Furthermore, the simplicity of this procedure allows an *in situ* separation of Cr species. However, coprecipitation is a time-consuming procedure and is often associated with a bad reproducibility. LLE has also been applied to chromium speciation in seawater both off-line and on-line when a flow injection analysis (FIA) is used. This treatment is based on the complexation of Cr(VI) complex followed by extraction with an organic solvent before analysis. SPE is also mentioned in several studies on Cr speciation in seawater in combination with FIA techniques. Preconcentration methods involving solid sorbents are considered better than LLE systems in terms of simplicity, rapidity and ability to obtain a high enrichment factor [VAS 00, HIR 06]. Due to its efficient preconcentration, detection limits obtained when SPE is used are low enough to determine Cr species concentrations in seawater but due to

their different physicochemical properties, the two Cr species are not easily simultaneously determined.

Modern analytical techniques such as HPLC-ICP-MS for chromium speciation have not been very often applied to seawater samples mainly because Cr species are present at the ng/L range in a highly complex matrix. Even with matrix separation/analyte preconcentration and the use of an ultrasonic nebulizer, detection limits are higher than those obtained by other methods [POS 96].

4.2.3.3. *Sediment analyses*

Analytical methods found in the literature for Cr speciation in sediment samples are reviewed by Amouroux *et al.* [AMO 11]. The number of studies dealing with the specific determination of Cr species is low for this type of matrix and most of the time, chromium bioavailability and mobility are still studied using the sequential extraction procedure [PEM 99, HUR 03]. The main difficulty in determining Cr(VI) in solid matrices arises from the possible interconversion of oxidation states of the different chromium species. It is now recognized that Cr(VI) extraction is facilitated by using alkaline conditions but unfortunately, Cr(III) is precipitated and cannot be determined simultaneously. Therefore, in most studies, only the most toxic species, hexavalent chromium, is determined [AMO 11].

From the review of Amouroux *et al.* [AMO 11], no study describing the specific determination of Cr species in marine biota is available.

4.2.4. *Inorganic and organic arsenic species*

4.2.4.1. *Introduction*

Arsenic is widely present in the marine environment as a result of both natural sources (mainly by leaching from geological formations) and anthropogenic sources (smelters, arsenic pesticides, herbicides,

etc.). The average concentration of total arsenic in seawater ranges from 1 to 2 $\mu\text{g/L}$ [NAK 00], whereas in marine animals the arsenic concentrations are in the order of few to 10 mg/kg of as [FRA 02].

This element occurs in different forms presenting varying toxicity. The admitted toxicity sequence for As species is in order $\text{As(III)} > \text{As(V)} > \text{monomethyl arsonate (MA)} > \text{dimethylarsinate (DMA)} > \text{organic forms}$ [PEN 74, JAI 00]. Contrarily to inorganic forms, organic forms such as arsenobetaine (AB), arsenocholine (AC), trimethylarsine oxide (TMAO), tetramethylarsonium ion (TETRA) or arsenosugars are toxic only with high doses [KAI 92, NEF 97]. Arsenic toxicity appears strictly related to its chemical form, and plants and animals are known to accumulate this element mostly as non-toxic organoarsenic molecules.

A traditionally used technique for As determination is the HG technique coupled to AAS that is used to separate the two inorganic forms As(III) and As(V) and the two simple methylated forms MA and DMA [BRA 73]. This method has since been widely used to study arsenic speciation but is mainly limited to aqueous matrices. The reader is invited to consult the review by Amouroux *et al.* [AMO 11] regarding the various techniques used for speciation analysis of As, which is itself based on many reviews published in the literature [GUÉ 99, GON 02, GOE 02, LEE 06].

Various specific detectors were used for arsenic speciation analysis, each providing a certain number of advantages and limitations in the results. The first major limitation is linked to sensitivity [AMO 11], e.g. detectors used in the past (FAAS, ICP-AES, etc.) are insufficient to determine traces present in natural samples. Another limitation is linked to the interferences, which may occur during the detection and which are particularly acute for the most used detector, i.e. ICP-MS. Even though this detection offers the best sensitivity as well as a multielementary capability, it suffers from polyatomic interferences in the case of arsenic analysis arising from the association of $^{40}\text{Ar}^{35}\text{Cl}$, which is particularly disruptive in the

analysis of marine samples when chromatographic conditions are not set to allow separation of chloride ions from arsenical species. This problem is now solved by the use of ICP-MS equipped of collision/reaction cell, allowing interferences removal [AMO 11]. In the following, we will focus on the most widely used techniques for As speciation in marine samples, from seawater to marine biota, illustrated by most recent papers.

4.2.4.2. Seawater analyses

Arsenate is the main arsenic form in seawater, while As(III), MA and DMA are present at lower levels [BRA 73, AND 78]. For As analyses, seawater samples are usually simply filtrated and stabilized with HCl, EDTA or hydrazine chloride to prevent oxidation of As(III) into As(V) before storage at 4°C [AMO 11]. This stabilization step remains however questionable as several studies have indeed demonstrated the risk of species interconversion with acidification [FRA 02]. Moreover, hydrochloric acid has to be avoided when ICP-MS not equipped with a collision/reaction cell is used (see above).

Analytical methods are either designed specifically for inorganic species (and sometimes methylated species) or for organic and inorganic forms (see Amouroux *et al.* [AMO 11] for details on existing techniques). The most popular technique is based on HG, enabling arsenic to be separated from the seawater matrix with the possibility to lower detection limit with the use of large samples. The method relies on the difference between the hydride-generating species (arsine), i.e. inorganic forms As(III) and As(V), mono- and dimethylated forms, and the non-hydride-forming species, i.e. organic species. Volatile arsines are produced by reaction with sodium borohydride in acidic media and flushed to a specific detector (AAS, AFS) by an inert gas. With a careful pH control, the hydride formation reaction enables to differentiate As(III) from As(V). Another approach is to coprecipitate As(V) with aluminum hydroxide ($\text{Al}(\text{OH})_3$), calculating As(III) from the difference between the total As content and As(III) content. It has been shown that arsenosugars can also

generate arsines [SCH 04a], therefore the use of HG should be questioned if some arsenosugars are present in water samples as algae decomposition products.

Another group of methods deals with electrochemical speciation of As(III) and As(V) in seawater and is based on As(III) electroactive species analysis and total As analysis after reduction of As(V) into As(III) by different reagents [VAN 07, DE 08]. In this case again, results for As(V) are obtained by difference. These methods have been in use for long but are now replaced by hyphenated techniques (e.g. coupling HPLC and GC with ICP-MS) in which a separation is coupled to a selective elemental detector, thus allowing full screening of the species present in the sample [AMO 11]. As highlighted by several reviews, HPLC interfaced with element-specific detection is the most popular method [GUÉ 99, GON 02, FRA 02, LEE 06] as it does not require a previous derivatization step to produce volatile species, which is not always feasible for all compounds. In the case of seawater analysis, anion exchange is usually chosen, allowing the separation of As(III), As(V), MA and DMA. However, the highly saline samples may deteriorate the separation processes and also the presence of chloride ion generates interferences by formation of ArCl when ICP-MS is used as detector. Dilution of the samples or pretreatment for removing the matrix is necessary [NAK 02] and therefore decreases the level of As species. There is also a risk that pretreatment modifies the original speciation in the sample [CAB 00]. As mentioned earlier, an alternative to sample dilution is the use of collision/reaction cell on new generations of ICP-MS (collision or reaction gases allowing to remove the interference created in the argon plasma).

4.2.4.3. Marine sediment analyses

For studies related to the mobility or bioavailability of As in sediments, the sequential extraction procedure is still often used [PRI 05, HUN 09], i.e. As is sequentially extracted from the easily extractable, carbonate, Fe-oxyhydroxide or hydrous ferric oxide, organic matter and residual fractions. Arsenic species

can also be specifically determined and methods cited in the literature for sediment samples are reviewed by Amouroux *et al.* [AMO 11].

Most of the time, the extraction method of choice for As species is with diluted phosphoric acid, which is followed by HPLC-ICP-MS determination [DEM 97, SAN 05]. Recoveries generally vary between 60 and 100% depending on physicochemical properties of sediments and are often improved by microwave heating. All studies pointed to the fact that the main problems encountered when using phosphoric acid as extracting reagent is a partial oxidation of As(III) to As(V) as well as a distortion of the ion-exchange based chromatographic separation, particularly for As(V). This is generally avoided by diluting the extracts before injection. In addition to As(III), As(V), MA and DMA, arsenosugars, arsenobetaine and traces of AC have been detected in marine sediments or in suspended particles. The occurrence of these compounds is directly linked to the presence of microorganisms, detritus of algae or marine organisms within the sediment [ELL 03].

4.2.4.4. *Marine biota samples*

The majority of studies on arsenic speciation in the field of marine environment are related to marine biota. Analyses concern almost all the different organisms encountered in marine ecosystems, i.e. algae, shellfishes, fishes, turtles, anemones, etc. [AMO 11]. Actually, apart from the two inorganic species and the methylated forms, a large variety of organoarsenicals have been identified [FRA 02, SCH 04b]. Food products are subject to intense monitoring of As species, i.e. edible fishes and seafood (shrimps, seashells, etc.), which are largely contributing arsenic to the European diet with possible implications on human health [BOR 07]. Arsenobetaine is the most commonly reported organoarsenical in marine animals. Arsenosugars are also found in marine algae and in marine animals such as scallops and have also been identified in fishes and mussels. Arsenosugars are assumed to be relatively non-toxic to animals and humans compared with inorganic arsenic species, but biotransformations of arsenosugars can

result in toxic arsenicals and As(V), As(III), MA and DMA can also be detected with lowest concentrations [FRA 02].

Extraction is one of the most critical analytical steps in arsenic speciation in marine organisms, owing to risks of losing the identity of the endogenous species. Various types of extracting reagents are used, e.g. tetramethylammonium hydroxide (TMAH) [ACK 99], alkaline alcohol [SLO 05], nitric acid [BRI 02], acetone [KIR 02], phosphoric acid [KUE 01], etc. They allow good recovery of As compounds with efficiency ranging between 80 and 100%, but the stability of the compounds during extraction is rarely checked.

The methods based on selective hydridation of inorganic As are now replaced by coupling of liquid chromatography (LC) with specific detectors. Different mechanisms available in LC enable us to separate different arsenic forms based on their chemical properties; the most commonly used are ion-pair chromatography, reversed-phase chromatography and ion-exchange chromatography (anionic or/and cationic). Applications of the approaches to different arsenic species are detailed by Amouroux *et al.* [AMO 11].

High-sensitivity detectors in use are ICP-MS, AFS and, in some cases, AAS, which is now rarely used owing to its lack of sensitivity. The last two detectors require on-line decomposition (UV or microwave) and volatilization (HG) of the separated compounds as the organoarsenicals do not generate hydride in classical hydridation conditions. Over the past few decades, the interest for AFS has increased, because of its low cost of investment and handling and its simplicity of use [AMO 11]. Examples of the above-mentioned techniques with their related performances are given by Amouroux *et al.* [AMO 11]. The reader will also find useful information in the reviews already mentioned in the refers to section 4.2.1.

4.2.5. Inorganic and methylated mercury species

4.2.5.1. Introduction

All the chemical forms of mercury are known to be poisonous, but the most toxic effect on human health is generally related to the

environmental transformations of inorganic mercury (IHg) into the toxic and biomagnified methylmercury (MeHg) form. Therefore, Hg speciation and particularly the assessment of IHg and MeHg in environmental matrices is an essential step in the interpretation of the Hg biogeochemical cycle in aquatic environments [STO 06]. The determination of mercury species from complex matrices present in marine ecosystems (e.g. water, sediments and biological tissues) is still considered a challenging task due to the very low levels of the endogenous mercury species in water samples, the extremely low relative concentrations of MeHg compared to IHg in sediments and the possible methylation/demethylation reactions occurring during the analytical procedures (water, sediment and biological samples). Therefore, Hg speciation and more precisely MeHg determination in environmental samples are faced with problems of non-quantitative recoveries as well as possible occurrence of artifact formation and transformations of MeHg during the sample preparation and separation steps [WIL 98]. Using isotopically enriched mercury species as tracers (determined by isotope dilution MS) has revealed that mercury species may be subject to abiotic transformations (i.e. methylation and demethylation), affecting seawater, biological materials and sediments, hence leading to potentially incorrect determinations. Multiple spiking species-specific isotope dilution analysis has been developed to overcome these problems [HIN 99, MON 08].

4.2.5.2. Seawater analyses

Three main forms of mercury are present in seawater: elemental mercury (Hg^0), inorganic mercury (Hg(II)) and organic mercury (MeHg and DMeHg). In seawater analysis, the sample pretreatment is a critical step owing to risks of adsorption onto the sample container and the occurrence of abiotic or biotic processes that could lead to a redistribution between chemical species. Water samples (filtered or non-filtered) are generally stored at 4°C in the dark after the addition of a small volume of ultra-pure concentrated acid. During the storage, Hg(II) can be lost by sorption on the bottle walls or by chemical processes, e.g. reduction and methylation [KRI 88], and reagents have

to be carefully selected to optimize the stability of Hg(II) solutions [AMO 11]. The stability of MeHg depends on the matrix [BAE 92], e.g. HNO₃ is effective for simple MeHg solutions but not for seawater that may be preserved with H₂SO₄ [JAC 88], HCl [COS 97] or frozen without acidification [BLO 89]. The stability of the mercury species during sample storage and the loss mechanisms were recently investigated [YU 03, PAR 05], studying various factors such as concentration of mercury species, matrix composition, container material, pH, temperature and light.

Volatile mercury forms (elemental mercury [Hg⁰], dimethylmercury [DMeHg]) may be also present in water in extremely low concentrations [COQ 97, AMO 98]; their determination requires large volumes of the samples. Samples should not be acidified owing to risks of rapid oxidation of the volatile species [PAR 05]. The analytes may be collected *in situ* using cryogenic traps and stored at low temperature (liquid nitrogen) until analysis in the laboratory [AMO 98] or can be purged onto a gold traps (for Hg⁰) and carbotrap (for DMeHg) [COQ 97]. If samples cannot be purged and trapped in the field, they should be collected in completely full glass bottles with Teflon-lined caps, as those species are lost rapidly from Teflon and polyethylene bottles [PAR 05].

Highly sensitive detection techniques are available for mercury (e.g. CV-AFS, ICP-MS) but speciation analyses require selective preconcentration procedures. SPE can be used for seawater analyses [KWO 00] to separate MeHg from Hg(II) by elution. GC is most often used for the separation of mercury species at environmental levels as the technique offers quantitative transfer to the detector and a better sensitivity. Direct analysis of MeHg in the form of MeHgX (X= Cl, Br) selectively extracted with organic solvents is carried out by GC with electron-capture detector [PUK 94]. However, the selectivity of this detector is low for mercury and it is necessary in most cases to derivatize the ionic mercury species in order to convert them to volatile forms, which are then separated by GC and detected by specific atomic detectors. This can be carried out by HG with sodium

borohydride (NaBH_4) with Hg(II) being transformed to Hg° while MeHg forms MeHgH ; speciation is difficult because the bond Hg-H is unstable but the half-life time of MeHgH is about 2 hr, which is sufficient for its determination [FIL 92]. HG can be therefore directly applied for sea and estuarine waters [RIT 94, TSE 00]. Ethylation is also used with sodium tetraethylborate (NaBEt_4), in which Hg(II) is transformed to HgEt_2 while MeHg forms MeHgEt ; this technique is motivated by the higher stability of MeHgEt as compared to MeHgH [AMO 11]. In on-line systems, the derivatization is directly connected to the analytical device and the automation of the analysis is possible reducing the risk of contamination and losses of the analytes. With the alkylation and derivatization, the volatile mercury derivatives are purged by means of a gas stream and trapped on adsorbent at ambient temperature [BLO 89, TSE 04] or on cryogenic trap [CEU 96, TSE 98, DEM 01, STO 04]. An enrichment factor of 50–100 can be achieved during the trapping process [DE 99]. The detection of the Hg species in on-line analysis of water samples has been achieved by AFS [BLO 89, RIT 94, CAI 96, STO 04, TSE 04], AAS [TSE 98], MIP-AES [CEU 96, SLA 00] and ICP-MS [AMO 98, TSE 00, DEM 01, BRA 04].

In off-line systems, the derivatization is independent and separated from the analytical technique used. Preconcentration is required in order to have low LODs before derivatization. Derivatization with Grignard reagent is used for different types of water [EMT 95]. Derivatized analytes are extracted with organic solvent, then separated by capillary GC and detected by MIP-AES. For the determination of MeHg in waters, extraction of MeHgCl in organic solvent can be applied. The extract can be analyzed, for example, with capillary GC-AFS [CAI 96]. Additional details on alternative techniques are given by Amouroux *et al.* [AMO 11] who review a wide range of methods with their respective performances.

4.2.5.3. Marine sediment analyses

Although contamination risks for bulk sediments during sample handling are less critical than for waters [MAS 98], sample

preparation still leads to possible drawbacks for mercury speciation. As for other types of speciation measurements, the sampling strategy depends on the objective of the study and is based either on sediment grabs collection for surface sediment samples [VAR 00, STO 04] or sediment cores. Sample oxidation has to be kept minimal during the treatment, especially for anoxic sediments [AMO 11].

Extraction for mercury speciation is based on various mild or diluted agents such as citrate buffer and extraction with various mixtures, e.g. dithizone/chloroform [HIN 93, HEM 95], dilute HCl/HNO₃ mixtures [DIE 01], etc., or alkaline digestion [RAM 01]; see Amouroux *et al.* [AMO 11] for further examples of reagents and extraction techniques (e.g. with supercritical fluids, distillation, microwave, etc.).

The question of the possibility of artifact formation of MeHg in sediments from inorganic mercury during sample preparation has been raised [HIN 99, ROD 03]; it was found that such species transformation may indeed occur in particular when distillation-based methods are used [HIN 99], depending upon the inorganic mercury amount present in the sample. The use of isotope dilution mass spectrometry (IDMS) has proven to be a powerful tool to check whether any interconversion is taking place. The use of Hg(II) and MeHg labeled with multiple isotopes allows discovering the error of the specific steps and their contribution to the overall transformations of a species known [WIL 98, HIN 99, ROD 03, RAH 05]. Artifact generation during mercury speciation analysis has been reviewed by Leermakers *et al.* [LEE 05].

Most of the separation and detection techniques described for water analyses are used for sediment extracts with some modifications [AMO 11], i.e. HPLC followed by AFS or ICP-MS detection allows us to quantify organomercury species at environmental levels in real samples [HIN 93, WIL98] but not Hg(II). Off-line and on-line derivatization methods (HG, ethylation) are also used for the determination of Hg species in sediment extracts by GC. Various detectors used with GC for analysis of environmental sediment

samples are quartz furnace FAAS [TSE 98], AFS [MOR 97, BOW 00, TSE 04], MIP-AES [DIE 01, ROD 98, LAN 04] and ICP-MS [WAS 98, JIT 04]. Some of the methods used for mercury species determination in sediments are summarized in the review by Amouroux *et al.* [AMO 11].

4.2.5.4. *Marine biota analyses*

Mercury is not uniformly distributed in organisms' tissues, which requires a consideration of the trophic level when adopting a sampling strategy for the determination of Hg species in marine biota. Samples are generally frozen immediately after the preliminary treatment and later directly analyzed in the laboratory or after freeze-drying [AMO 11]. The analysis of biological tissues is less affected by artifacts and interferences during extraction procedures and quantification as compared to sediments. Quantification requirements are easy to achieve owing to the high amounts of MeHg usually present in biota samples. Similar methods as for sediments can be applied to extract the mercury forms [BAE 92]. Alkaline or acid digestion at ambient temperature or with conventional heating can be accelerated by means of ultrasonic [BLO 89, DON 04, PER 05] or microwave [TSE 04, DIE 01] treatment. The distillation is also used for MeHg extraction in biological tissues [WIL 98]. Biological tissue extracts can be analyzed by similar methods as the ones described for waters and sediments. Some of the numerous methods used for mercury species determination in biological tissues are summarized by Amouroux *et al.* [AMO 11].

4.2.6. *Butyltin and other organotin species*

4.2.6.1. *Introduction*

The marine environment has been and still is most impacted by organotin compounds, in particular TBT, which has led to a widespread contamination of marine ecosystems owing to its high toxicity, particularly in coastal and harbor areas [DON 01]. TBT is an efficient agent to prevent fixation of aquatic organisms on the hull of

ships but its use and toxicity have caused extensive damage to non-target organisms, e.g. shell deformations in oysters [ALZ 91, ALZ 98], sex changes (imposex) in whelks [BRY 86, GIB 87] and harmful effects in marine life at very low concentrations [EVA 99]. Consequently, several countries have implemented national legislation from the early 1980s in order to limit (and sometimes ban) the use of TBT in antifouling paint. The IMO called for an international ban of TBT in antifouling paints by the January 1, 2008 through the AFS Convention (Convention on the Control of Harmful Anti-fouling Systems on Ships). As mentioned in Chapter 3, the EU Directive 2008/105/EC, complementing the EU Framework Directive, has set EQS for a range of substances including TBT (as TBT-cation) for inland and other surface water. There is however no limit recommended for sediments or biota. Similarly to other chemical species, the different toxicity of individual organotin species requires that analytical techniques are able to determine various substances, and a huge range of methods has been developed over the past 30 years.

Organotin compounds have been demonstrated to be ubiquitous in the marine ecosystem, but their species repartition can be subject to transformations in water, with exchanges taking place between different environmental compartments (water, suspended matter, sediment). These transformations may lead to enhanced availability to filtering or sediment-feeding organisms, leading to bioaccumulation in the food chain and impacts of this compound on large biological systems in acting as endocrine disrupters. Consequently, TBT is frequently measured in organisms from higher levels of the marine trophic chain such as tuna [KAN 96, UEN 04], whales, dolphins [ABE 00], seals [BEL 00] and birds [KAN 98].

As readily described for other organometallic species, most analytical techniques developed for organotin speciation usually combine separation techniques such as HPLC [HIL 00] or GC [YAN 01] with a specific detection mode such as Pulse Flame Photometric Detector (PFPD) [SZP 96], AAS [CAI 93b], AES [JIM 97, AGU 01] or ICP-MS [HIN 95, AGU 01, YAN 01]. With a derivatization step to yield volatile species, e.g. by Grignard reaction,

HG or ethylation [MOR 00], coupling of GC to ICP-MS is achieved (which has been shown to be one of the promising techniques for organotin analysis because of its multielement capability and the possibility of isotopic information and its high sensitivity) [YAN 01, AGU 01].

4.2.6.2. Seawater analyses

Organotin levels in marine waters are, in general, extremely low (below theng/L level), hence a high risk of contamination at the sample handling and treatment steps prior to the determination. For water samples, analyses are generally performed after filtration at $0.45\ \mu\text{m}$ to determine organotin concentrations both in dissolved and particulate phases. The toxicity of organotins is generally referred to their concentration in the dissolved phase but they are also determined in the particulate phase because of the tendency of TBT to be adsorbed on particles. Analyses of non-filtered water provide results that are not really representative of the real contamination due to the difference of performances of the extraction techniques used [AMO 11] and in addition these analyses do not yield easily comparable results. Storage of collected samples is one of the most critical aspects of the whole analytical procedure because sample degradations may occur, affecting the quality of the final determination. Organotin compounds are stable for more than 4 months in sea and fresh water samples, filtered and acidified at pH 2 with HCl and stored in the dark at 4°C [AMO 11]. However, the stability of butyltin species is less easily achieved in non-filtered samples containing high amount of suspended matters, probably due to possible interferences with particulates and/or microbial communities [QUE 96b].

Organotin analyses at low concentration levels require extremely sensitive and selective techniques based on derivatization, separation and detection steps, i.e. GC or LC coupled with highly selective and sensitive detectors, such as MS [WHI 98, IKO 02], AAS, flame photometry [LEG 03] and flame ionization detection [MIL 02]. Among these, ICP-MS in combination with GC provides excellent

sensitivity, selectivity, multielemental [AGU 01] and multi-isotope detection capabilities [RUI 01, YAN 02]. Few examples of this technique and the associated detection limits are presented by Amouroux *et al.* [AMO 01].

4.2.6.3. Marine sediment analyses

Organotin compounds in coastal waters rapidly adsorb onto suspended matters and settle. Trapping of the compounds in sediments leads to long-term storage, which may last from 1 year to more than a decade [CRA 86]. After sediment collection, different treatments are used for storage (freezing, wet storage at 4°C) and drying (air-, frozen, oven-), which are suitable to preserve the stability of TBT at least for more than 4 months but MBT and DBT are often subject to variation (mostly losses). In case of long-term storage, freeze-drying is a better compromise [QUE 90]. Higher organotin concentrations are linked to fine sediment particles, hence sieving of samples at 2 mm and correlation to grain-size particles may simplify comparison of results.

Most sample preparation techniques for butyltin speciation in solid matrix combine different complex preparation steps using stirring [KRO 89, GÓM 95], sonication [CEU 94, GÓM 95, PEL 00] or supercritical fluid extraction (SFE) [BAY 94]. Open microwave assisted digestion is also increasingly used as a rapid and efficient method for sample decomposition [DON 95, ROD 96]. IDMS represents a good alternative method to avoid losses during the sample preparation procedure, i.e. if the equilibration of the spike and the analyte is fully achieved, IDMS is theoretically capable of compensating for non-quantitative sample preparation procedures [ROD 02]. Some applications for OTC determination in marine sediments are summarized by Amouroux *et al.* [AMO 11].

4.2.6.4. Marine biota analyses

Organotins are determined in biological tissues, particularly in benthic organisms, in order to evaluate the bioconcentration in the

food web. Similarly to other environmental analyses, sampling strategies have to be representative of the real distribution of the different species and the analytical method should be sufficiently accurate and precise to measure low concentrations in very low mass samples [AMO 11]. Extraction has to be carried out in such a way that the integrity of the speciation is preserved. Organotin compound extraction from biological samples is generally performed with acetic acid [ROD 96]. Microwave extraction allows solubilization of biological tissues and the complete extraction of butyltin compounds in a short extraction time (3 min). Further to the extraction step, organotins are derivatized similarly to what is described for sediment samples, i.e. Grignard reaction or HG, as well as aqueous ethylation [RAP 94, TSE 99, GAR 00]. IDMS has also been developed for TBT analysis in biological tissues [MON 03].

4.3. Organic micropollutants

4.3.1. Introduction

Organic micropollutants, singly or in combination, can exert adverse effects within the aquatic environment and, for compounds that are persistent and that bioaccumulate, potentially also on human consumers of seafood [LAW 11]. Most organohalogen pollutants have been of environmental concern for nearly 50 years, e.g. DDT was identified as one of the first persistent pollutants found all over the world, causing harm to the environment [CAR 62]. Other organochlorine pesticides such as HCH, HCB, toxaphene, etc., have been shown to be equally persistent. Among the “dirty dozen” of pollutants banned under the Stockholm Convention (aldrin, chlordane, dieldrin, endrin, heptachlor, hexachlorobenzene, mirex, toxaphene, DDT, PCDDs, PCDFs, and PCBs), the first nine compounds belong to the group of organochlorine pesticides. PCDDs, PCDFs and PCBs have different sources [LAW 11]. This section provides a short description of current analytical methods that have been reviewed by Law *et al.* [LAW 11].

4.3.2. Polychlorinated biphenyls

4.3.2.1. Introduction

PCBs were produced and used in large volumes from the late 1940s onward. PCBs were prepared industrially by the chlorination of biphenyl (the most well-known are Aroclor, Clophen, Kanechlor, and Phenoclor, graded and marketed according to their chlorine content, e.g. Aroclor 1232 contains 32% by weight of chlorine). PCB compounds were widely used in transformers, capacitors, hydraulic fluids and as plasticizers in paints, plastics and sealants, but the production in Western Europe and North America ceased in the late 1970s [LAW 11] due to concerns about the environmental impact of PCBs. Theoretically, 209 individual PCB congeners may be produced, depending on the number and position of chlorine that is substituted onto the biphenyl amount. However, only a limited number of PCBs have been produced from the conditions involved during the industrial process. Individual congeners are generally named using a number from 1 to 209 often prefixed with “CB” (see a review by Mills *et al.* [MIL 07]). Monitoring programs focus on a limited number of the 209 PCB congeners, e.g. ICES considers the CB28, 52, 101, 118, 153, 138 and 180. The most toxic PCB congeners are the so-called “dioxin-like” PCBs, i.e. the four non-*ortho* (CB81, 77, 126 and 169) and eight mono-*ortho* PCBs (CB105, 114, 118, 123, 156, 157, 167 and 189). The main historical sources of PCBs to the marine environment included energy production, combustion industries, production processes and waste (landfill, incineration, waste treatment and disposal). High concentrations are still found in marine sediments due to their persistent nature, in particular around industrialized and urbanized areas. In addition, PCBs are hydrophobic and have the potential to bioaccumulate, particularly in lipid-rich tissues such as liver [LAW 11].

4.3.2.2. Analytical methods

Analytical methods for PCBs used in environmental monitoring have been reviewed by several authors, e.g. Smedes and De Boer [SME 97], Wells and Hess [WEL 00], Muir and Sverko [MUI 06] and

more recently Law *et al.* [LAW 11]. Methods' performances had to improve owing to the decreasing environmental concentrations of PCBs; these methods consist of three main steps: extraction, clean-up and instrumental analysis.

4.3.2.2.1. Extraction methods

Classical Soxhlet extraction methods can be applied to sediment and biota samples. Soxhlet is time-consuming with extraction times of 3–12 hr and uses large volumes of solvent (100–500 mL) as compared to other newer alternative techniques. The method can however be used for large sample sizes (up to 200 g of sediment and 100 g of biota) and it is not matrix dependent [LAW 11]. Usually, polar solvents such as dichloromethane or polar/non-polar mixtures are used, although for health and safety reasons dichloromethane should be avoided. An alternative solvent, which is as efficient as dichloromethane, is methyl-*tert*-butyl ether. Less time- and solvent-consuming procedures have been investigated such as SFE and pressurized liquid extraction (PLE). In the SFE approach, a supercritical fluid such as ammonia, methane or carbon dioxide is used for the extraction rather than a solvent, and carbon dioxide is the most common supercritical fluid [LEE 94, TON 95]. An advantage of SFE is that extracts are cleaner and additional clean-up steps are not required. A drawback is that only a small sample size (<5 g) can be used, with increased detection limits of the method and a poor reproducibility as compared to Soxhlet extraction [WEL 00]. Most promising is PLE in an automated mode [SPO 03, GÓM 02]; this method is matrix independent and is used widely for the extraction of POPs in environmental samples [LAW 11].

4.3.2.2.2. Clean-up procedures

Clean-up is essential, especially if concentrations are low. It enables to remove interfering substances, e.g. lipids in biota samples or sulfur in sediments. Various techniques are used, e.g. GPC, adsorption column chromatography, dialysis, sulfuric acid treatment, saponification or a combination of these [WEL 00, MUI 06], details of which are given by Law *et al.* [LAW 11]. It is often necessary to apply

to different clean-up methods in combination such as silica gel and sulfuric acid. Concentrated sulfuric acid is often very useful to clean the extracts and ensures clean chromatograms; it is however an aggressive reagent that leads to degradation of some organochlorine pesticides such as dieldrin and endrin. For non-*ortho* PCBs, which are normally found at substantially lower concentrations compared to the *ortho* and mono-*ortho* PCBs, a separation step is required to allow for extract concentrations, using various techniques such as activated carbon, adsorption columns, charcoal, etc., with a preference for HPLC [HES 95]. This is further discussed by Law *et al.* [LAW 11].

4.3.2.2.3. Preconcentration

Concentration steps are required after extraction prior to GC analysis, avoiding evaporation to dryness. Until recently, rotary-film evaporator was the most common concentration method, carried out at low temperature and under controlled pressure conditions in order to prevent losses of the more volatile PCBs [LAW 11]. New, less time-consuming techniques are Turbo-Vap and Syncore concentrators that can be used to simultaneously reduce the solvent volume of a number of samples. When reducing the sample to the required final volume, solvents can be removed by a stream of clean nitrogen gas. Suitable solvents for injection into the GC include *n*-hexane, *n*-heptane, toluene and *iso*-octane.

4.3.2.2.4. Detection and quantification

GC coupled to a suitable detector is a method of choice for PCBs analyses, using a wide range of stationary phases [VER 07] and various settings (column length and internal diameter), examples of which are given by Larsen *et al.* [LAR 95] and Law *et al.* [LAW 11]. Detectors most used for PCB determinations include ECD, LRMS (low-resolution mass spectrometry), ITMS/MS (ion trap mass spectrometry), time of flight MS and HRMS. Historically, ECD has been the most commonly used detector for the analysis of *ortho*-PCBs, however MS is nowadays more commonly used. MS techniques are more selective and $^{13}\text{C}_{12}$ -labeled internal standards can

be used. Next to the conventional GC-MS, the use of ion-trap with its MS² option – i.e. increased selectivity – is receiving increased attention [LAW 11]. GC-ITMS is a less-expensive alternative to HRMS, which is commonly used to determine PCDD/Fs and as such also ideally suited for all CB groups [EPP 04].

4.3.3. Polybrominated diphenyls ethers

4.3.3.1. Introduction

Polybrominated diphenyl ethers (PBDEs) have been used as flame retardants (FRs) in electrical equipment, textiles and furniture since the 1970s up until they were banned in Europe in the 2000s [LAW 11]. The bromine industry has gradually terminated the production of all PBDE mixtures in North America. Commercial PBDE mixtures are classified according to the degree of bromination. Historically, these compounds were released in the environment during their production and product manufacturing. Since their ban, main sources remain from the disposal of products that contain these chemicals, as well as from leaking out of treated material in indoor environments [JON 05, JOH 09]. In the marine environment, the major pathway for PBDEs is via atmospheric transportation. Other possible pathways include direct discharge from point sources such as storm waters and waste water.

PBDEs consist of two phenyl rings, each containing up to five bromine atoms. Among the 209 possible PBDE congeners, only a limited number (~20) is used in FR technical mixtures [BAL 92]. Most laboratories analyze tetra- to hexa-PBDE congeners, which are thought to be the most toxic and likely to bioaccumulate in biota (in particular, the BDE47, 99, 100, 153 and 154 compounds) while BDE209 can be predominant in sediment samples. PBDEs are hydrophobic and therefore tend to associate with particulate material, accumulating in sediment, in particular, in the presence of high organic carbon content [LAW 08].

4.3.3.2. Analytical techniques

Analytical methods for the analysis of PBDEs in environmental samples have been reviewed by Covaci *et al.* [COV 07] and Law *et al.* [LAW 11]. Techniques used for PCB analyses may also be applied for PBDE determinations.

4.3.3.2.1. Extraction methods

Extraction methods used for PCBs can be applied to PBDEs for both sediment and biota, i.e. Soxhlet and PLE. SFE has also been used with a poorer reproducibility as compared to Soxhlet [COV 03]. Hexane/acetone mixtures or toluene (particularly for BDE209) have been shown to give the best recoveries for the extraction of PBDEs [DE 01], except for BDE209 that can be lost during PLE through adsorption onto the extraction system tubing [COV 03].

4.3.3.2.2. Clean-up procedures

Owing to the low environmental concentrations, suitable clean-up is essential to removing interfering compounds that may be present in the extract. Techniques used for PCBs can often be applied to PBDEs [LAW 11]. The choice of the method will be influenced by the selectivity and sensitivity of the final measurement technique and also by the extraction method employed. A second clean-up step may be required to separate PBDEs from other organohalogenated compounds. Lipid removal techniques such as saponification are used for biota, but it can result in the degradation of the higher brominated PBDEs and therefore is rarely used. PLE methods with on-line clean-up previously described for PCBs can also be used for PBDEs [BJÓ 06]. Similarly to PCBs, sulfur should be removed from sediment extracts, in order to reduce interferences and to protect the detectors. Methods described in section 4.3.2 can also be used for PBDEs [LAW 11]. Concentration techniques used for PCB analysis can also be used for PBDEs.

4.3.3.2.3. Detection and quantification

Instrumental techniques for the analysis of PBDEs have been reviewed by Stapleton [STA 06] and Law *et al.* [LAW 11]. The most

common injection techniques used for PBDEs are splitless, on-column and programmable temperature vaporization (PTV). Various capillary columns are used for PBDE analysis [KOR 05a]. BDE209 can suffer degradation during injection and precautions are required as described by Korytár *et al.* [KOR 05b]. GC-MS is the preferred technique for the quantification of PBDEs in environmental samples, using either electron impact or electron capture negative ionization (ECNI) [STA 06]. Degradation of BDE209 during analysis can be avoided by using an LC method instead of GC [ABD 09].

4.3.4. Emerging contaminants

4.3.4.1. Other brominated FRs

Other BFRs are released into the (marine) environment besides PBDEs, partly due to the termination of their production [LAW 11]. The highest production is of TBBP-A among all BFRs, which is present in the environment (mainly in waters) in relatively low concentrations (usually determined by derivatization followed by GC-MS or LC-MS). HBCD is another BFR that is produced in large volumes, and mainly present in biota (generally determined by GC-ECD or GC-MS). There are a number of other BFRs that are being introduced to serve as alternatives for the PBDEs. These may not have come yet to detectable environmental concentrations, but some may have the potential to do so, e.g. bromophenols, intermediates in FR formulation such as bromoanilines, and their brominated and non-brominated by-products such as bromoanisoles, bromotoluenes, bromoalkanes and 1,5,9-cyclododecatriene. Further details and references are given by Law *et al.* [LAW 11].

4.3.4.2. Phosphorus-based FRs

Phosphorus flame retardants (PFRs) are often proposed as alternatives for BFRs. Three main groups are distinguished: inorganic, organic (non-halogen) and halogen-containing PFRs, the latter two groups containing hundreds of compounds. Examples of compounds frequently reported in the environment are triphenyl phosphate (TPP), *tris*(2-chloroethyl) phosphate (TCEP) and *tris*(chloropropyl)

phosphate (TCPP), albeit few data are available for the marine environment [LAW 11]. These compounds are generally analyzed by, for example, SPE or microwave-assisted extraction (MAE) and analysis of the extracts is carried out by GC-MS or GC-ICPMS [GAR 09].

4.3.4.3. *Chlorinated paraffins*

Chlorinated paraffins (CPs) are also released in the environment, although they received less attention than PCBs from environmental analysts. These compounds are divided into short-chain, medium-chain and long-chain chlorinated paraffins. Analytical methods for CPs in environmental matrices have been reviewed by Tomy [TOM 10]. Soxhlet extraction and PLE are the most commonly used methods for CP extraction from biota and sediments, while various techniques have been used to remove coextracted lipids from extracts, e.g. sulfuric acid treatment or sulfuric acid-silica gel column chromatography [INO 05]. Adsorption chromatography on Florisil, silica and alumina has been used to separate CPs from interfering compounds [THO 06]. ECD is not much in use as detector for single-column GC analysis of CPs, which are rather determined by techniques such as HRMS [TOM 97, MOO 04].

4.3.4.4. *Siloxanes*

Siloxanes are issued from silicone products. Owing to their properties, these compounds are widely used in paper and mold release agents, medical and cosmetic products, polishes, coolants and dielectric fluids for electric systems and personal care products [LAS 05]. The most common forms of siloxane polymers are fluids, gels, elastomers and resins. Silicone fluids are usually straight chains of polydimethylsiloxanes (PDMS) intensively used as antifouling agents on ships. Although not halogenated, siloxanes may show a persistent and bioaccumulating behavior in the environment [LAW 11]. A review of analytical methods used to measure siloxanes has been made by Varaprath *et al.* [VAR 06]. PDMS is commonly analyzed with ICP-AES by measuring the silicon (Si) concentration in the sample [CAR 95, FEN 97] or by GPC and ICP-AES coupling [VAR 06]. The more polar siloxanes can only be separated by GPC and measured by GC if they are derivatized. Another option is

measuring them with HPLC [DOR 94]. The advantage of supercritical fluid chromatography (SFC) is that it extends the range of GC and yields nearly identical resolution and selectivity, while it is also possible to measure volatile and thermally labile compounds [VAR 06]. Other detection methods used for qualitative analysis are infrared (IR) and nuclear magnetic resonance (NMR). Particularly, NMR is widely used for the characterization of silicon compounds [VAR 06].

4.3.4.5. *Miscellaneous*

Many emerging organic contaminants are found in marine environmental matrices, in particular concentrating in sediments and marine organisms, generally analyzed by GC-MS or LC-MS [LEP 09], e.g. pharmaceuticals such as metropolol, diclofenac, sotalol, sulfamethoxazole and tramadol, and illicit drugs such as benzdazepines, cocaine and δ -9-tetrahydrocannabinol in surface water samples. Some of these compounds may be identified by passive sampling techniques [GRE 09, SÖD 09].

4.3.5. *Organohalogens in water*

4.3.5.1. *Introduction*

Organohalogen compounds are commonly detected in seawater, originating from remote sources, supplemented by deposition from the atmosphere and exposing biota to these contaminants. The highest concentrations in seawater are of HCH isomers (despite restrictions in production and use over the last decade); other halogenated POPs are one to three orders of magnitude lower (lower pg/L to sub pg/L range). Data on chlordane, heptachlor, mirex and toxaphene concentrations in marine waters are not available. Due to their high lipophilicity (and existing production and application restrictions, at least in Europe), water is not likely to be a primary monitoring matrix for these compounds. The same is true for PCDD and PCDF, which are expected to be found only in the low fg/L range [LAW 11]. The water phase is the favored matrix for polar compounds having log K_{ow} values less than 5 while non-polar pollutants adsorb to solid particle (SPM or sediments) and thereby are removed by sedimentation faster

from the water column. Law *et al.* [LAW 11] highlight that the water phase has some specific advantages over sediments or biota when surveys focus on the spatial distribution of pollutants, investigating input sources, transport paths and the ultimate fate of pollutants. The water phase is a “primary” compartment and does not require normalization, while in sediments and biota, enrichment and other factors depend on intrinsic matrix parameters. The water phase, unlike sediments and biota, has a short response time, and there is hardly any time integration, which enables to detect peak events at the expense, however, of a higher natural variability. In long-term monitoring, this may require high sampling frequencies with high associated costs. However, this is often less problematic in open oceans due to the slow dynamics of large water bodies. In coastal areas and estuaries, a correlation to salinity may “normalize” for variability caused by freshwater mixing. A different approach to overcome variability by high dynamics in the water and to increase time integration is to use passive samplers [LAW 11].

4.3.5.2. Analytical methods

Organohalogen concentrations are very low in the marine environment (low pg/L to low ng/L range) and analytical procedures hence always require an extraction (sometimes followed by a clean-up step) and enrichment step and a very selective chromatographic/mass spectrometric determination step [LAW 11]. “Classical” organohalogenated POPs are relatively non-polar (with log K_{ow} values between 3.5 and 8), are quite stable and can be evaporated without destruction, and they can thus be analyzed by gas chromatographic techniques. LLE using a non-polar solvent such as hexane or pentane is a suitable extraction and enrichment method. For more polar and non-volatile compounds such as PFOS, LC by means of tandem mass spectrometry is the method of choice. SPE is the preferred extraction and enrichment technique [LAW 11]. These extraction options (LLE, SPE) and determination techniques (GC-MS and HPLC-MS/MS) allow for a wide range of different compounds to be analyzed.

4.3.5.2.1. Extraction methods

Owing to the low organohalogen concentration ranges, large volumes of 10–100 L (or more) have to be sampled and extracted.

This may lead to blank problems or insufficient selectivity due to matrix background. “Classical” organohalogen pesticides (such as DDT, HCH, HCB, dieldrin, endrin, etc.) can be extracted and enriched from seawater by means of LLE using hexane or pentane [LAW 11]. For volumes larger than 200–1,000 L, SPE using large extraction cartridges filled with XAD resins have been successfully used for PCBs, DDT and PAHs, but not for HCH [SCH 95]. For the analysis of polar compounds by HPLC-MS/MS, smaller sampling volumes (1–30 L) and smaller SPE cartridges (1–3 g of resin) are used. The reason for this downscaling is the fact that polar compounds often occur at higher concentrations [LAW 11].

4.3.5.2.2. Clean-up procedures

Clean-up is essential prior to GC-MS analysis for protecting the GC injector and column from polar, non-volatile matrix compounds such as fatty acids, esters, alcohols and others, and for removing matrix compounds that can overlap with the signals of the analytes. In general, clean-up techniques similar to those described for PCBs or PBDEs can be used for organohalogen pesticides (see above sections). A simpler clean-up is used (e.g. small silica gel column) for seawater in comparison to more complex biological tissues or sediments matrices. The effectiveness needed for the clean-up depends on the detection technique used and on the concentration range. Less selective techniques such as ECD require a more stringent clean-up than MS/MS detection, and with an LOQ of 100 pg/L less clean-up may be necessary than with a range below 5 pg/L [LAW 11]. Larger amounts of silica gel may be required for estuarine water samples or samples from a region with high biological activity (algal bloom). For special applications, even an HPLC preseparation step may be necessary [PET 88]. The activity of the silica gel and the elution step has to be carefully controlled to avoid losses or coelution with matrix compounds, especially for medium polar compounds such as HCH or dieldrin. Samples analyzed by HPLC-MS/MS mostly do not require a clean-up step because the technique is quite robust and highly selective [LAW 11].

4.3.5.2.3. Detection and quantification

Most organohalogen pesticides can be analyzed using procedures used for PCBs (see section 4.3.2.2), i.e. GC with ECD. This method used with 10L samples allows us to obtain high limits of quantification for HCH and DDT metabolites. GC-MS became a method of choice in the 1990s, offering same levels of limits of quantification at an affordable cost, as well as a better selectivity (allowing simpler clean-up) and the suppression of background “noise” from matrix constituents. In addition, the range of multicomponent analysis has been extended. For example, simultaneous analysis of PAHs and chlorinated hydrocarbons is possible in one sample extract and one GC-MS analysis [LAW 11].

4.3.6. Polycyclic aromatic hydrocarbons

The determination of PAHs in samples from the marine environment using chromatographic techniques has been reviewed by Poster *et al.* [POS 06]. The main findings of that review, still up-to-date and relevant, have been updated by Law *et al.* [LAW 11], with discussions about GC-MS performances. Two-dimensional GC (GC \times GC) has also been applied successfully to a number of complex mixtures of isomers and congeners of environmental contaminants, including dibenzo-*p*-dioxins and furans, PCBs and polybrominated diphenyl ethers, and oil fingerprinting studies [ARE 07]. Unresolved complex mixtures (e.g. present in petroleum-contaminated sediments) can hardly be analyzed using traditional GC methods; however, their composition is of interest and their toxicity of possible concern [GOU 90]. Advances in GC \times GC using a variety of stationary phases enabled to improve such analyses [FRY 03].

The WFD (see Chapter 3) has yielded interest and requirements for the determination in a wide range of organic contaminants, and improved multiresidue analytical methods [LAW 11]. A method for the determination of 36 priority substances, including 8 PAH compounds from naphthalene to indeno [1,2,3-*cd*]pyrene, has been

developed, using SPE with Strata X cartridges [BAR 09]. Naphthalene was determined using GC-MS, and all eight PAHs using HPLC with fluorescence detection. Other compounds were determined using either GC-MS or HPLC-MS/MS. Using a new extraction technique known as stir bar sorptive extraction (SBSE), Huertas *et al.* [HUE 07] developed a method for 24 priority substances within the WFD, including four PAHs from benzo [*b*]fluoranthene to benzo [*ghi*]perylene. Other approaches included the use of a molecularly imprinted polymer as a solid-phase adsorbent for the quantitative enrichment of PAHs in coastal sediments [KRU 09] with the determination of PAHs (eight compounds from acenaphthene to benzo [*a*]pyrene) by fluorescence spectrometry. The general application of passive sampling devices for sample collection also showed a number of advantages that are further exploited nowadays (see Chapter 3). Deployed within sediment samples, these devices can also indicate concentrations of contaminants within sediment pore waters, i.e. those bioavailable to sediment-dwelling organisms that uptake from that matrix rather than by ingesting sediment particles [LAW 11].

4.4. Nutrients

4.4.1. Introduction

Eutrophication, mostly driven by increasing organic matter and nutrient loading, has been identified as one of the major threats to marine ecosystem health since the early 1970s [RYT 71]. International nutrient monitoring guidelines in North America and Europe [EPA 01, EUR 03] therefore address, *inter alia*, eutrophication as a process that affects ecosystem health in brackish and coastal waters [ROC 11]. In Europe, management strategies and related actions target the sources of nutrients that cause eutrophication [ELL 99, DE 02]. The most rapidly growing inputs of anthropogenic nitrogen to the marine ecosystem, both in terms of loading and geographical scale, are atmospheric deposition and groundwater inputs [PAE 99]. With the increasing understanding of eutrophication, different drivers of nutrient enrichment and their impacts on the global

health of the marine ecosystem are identified [VAQ 08, RAB 09, DAV 10], which condition the design of new marine nutrient monitoring strategies, including the choice of parameters, sampling and analytical techniques, all of which being a function of the target matrices and quality guidelines that are crucial to underpin the traceability of measurements [ROC 11].

4.4.2. Nutrient monitoring

Nutrient concentrations have a direct impact on phytoplankton growth and hence on eutrophication, whereas chlorophyll *a* and oxygen depict the reaction of a system impacted by eutrophication (increase in primary production and related decrease in oxygen levels). Ecosystem quality parameters include ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) and soluble reactive orthophosphate concentrations (PO_4^{3-}). These compounds, along with silicate (Si(OH)_4), are commonly designated by the generic term “nutrients”, and are an integral part of nutrient monitoring programs since both their quantities and relative availability (nutrient ratios) have a direct bearing on phytoplankton growth [ROC 11]. Total phosphorus, including dissolved organic phosphorus, and total nitrogen, including dissolved organic nitrogen and particulate organic nitrogen, are also included as measurements because of their importance to ecosystem function analysis and nutrient budgets. However, in the context of ocean monitoring, quantitative measurements and temporal variability of nutrients are not as relevant as in shelf seas that have a continental land-mass influence, but they may be indicative of continuous and sustained pressure, the analysis of which requires long-term monitoring and provision of high-quality datasets, particularly in oligotrophic regions [SCH 03, SCH 04].

The comparability and traceability of nutrient data in the global ocean is a matter of crucial importance, particularly in the context of global environmental change [ROC 11]. The need for intercomparability in order to comprehend ecosystem functional resilience in response to human pressure is much required since the global biogeochemical cycles of nitrogen and phosphorus closely

link food security, sustainability, biodiversity and ecosystem health [VIT 97, COR 09]. However, the issues of data quality, comparability and traceability are insufficiently discussed [QUE 08] in both the WFD and, by extension in the Marine Strategy Framework Directive (see Chapter 1). This is generally accepted as a serious shortcoming of available nutrient monitoring programs, in spite of the consensual agreement that monitoring has to be designed, necessarily, in a way that permits long-term trends or shifts in concentrations to be detected over vast geographical areas [ROC 11].

The analytical determination of nutrient concentrations in saline matrices is affected by a suite of specific difficulties, e.g. both nitrate and nitrite are normally determined as a total ($\text{NO}_3^- + \text{NO}_2^-$), which must be carried out by ensuring complete reduction of nitrate to nitrite. Nitrite must also be determined in isolation, sometimes after the removal of the cadmium reduction process from the system, but normally with different methodological precision and accuracy (e.g. [PAI 94]). This makes the process intrinsically dependent on the technical and operational proficiency of the analyst and the efficiency of the reduction step of nitrate to nitrite, which cannot be fully validated owing to a lack of effective certified standard reference material of nutrients [ROC 11].

4.4.3. Analytical methods

4.4.3.1. Analytical techniques

In the 1970s, nutrient determinations were mainly carried out by manual colorimetric methods [STR 72]. The development of automated batch analysis in the form of continuous flow analysis (CFA) systems enabled us to not only process a larger number of samples or determine simultaneously the full suite of nutrients from a single aliquot, but also to stabilize analytical conditions and hence improve measurement reproducibility. Unsegmented FIA developed very quickly for several applications [RUZ 08], but was faced with sample contamination risks due to the lack of flow segmentation, making this technique inappropriate for seawater samples with very

low nutrient concentrations. FIA is, nevertheless, still used for porewater nutrient analysis, for instance [ROC 11]. Most methods for nutrient analysis are based on manual procedures that have been continuously refined over the years (e.g. [STR 725, PAR 84, GRA 99]), see a review by Wurl [WUR 09]. Their use in marine monitoring programs and their operational limitations have been documented by, e.g., Kerouel and Aminot [KER 87], Kirkwood [KIR 96], ICES [ICE 05] and HELCOM [HEL 08], and series of laboratory performance studies have been performed [ICE 77d, KIR 91, GLA 97, WIL 00]. With few exceptions (e.g. [EPA 01]), however, there are no prescribed analytical methods in the regulatory framework for nutrient monitoring programs [ROC 11]. This is partly due to the fact that methods used by different laboratories in different countries are often selected according to the specific nature of the seawater matrix of the monitored area (e.g. salinity, pH, amounts of suspended solids, organic matter, etc.) and that procedures also depend upon the available laboratory equipment, technician skills and/or sampling and laboratory conditions [ROC 11]. Prescriptive methods are actually critically considered in marine nutrient monitoring owing to assumptions regarding their method performance characteristics that are actually directly dependent on the sample solution matrix and the analytical chain performance. For example, OSPAR [OSP 97] provides detection limits for phosphate, nitrate + nitrite, nitrite, ammonium and silicate, while EPA [EPA 01] suggests not only a range of detection limits, but also makes reference to specific analytical protocols with several alternatives in each case. The prescription of analytical protocols under some regulatory frameworks has often more to do with the fact that previous internal quality control (IQC) rules have been developed for a standard methodology, and this way their direct application by stakeholder institutions or individuals would benefit from existing QA. However, unless each laboratory conducts a rigorous quality control study with the method of choice on the matrix it is analyzing, with the analytical equipment available and the laboratory environment in which the analysis is effectively to be conducted, rarely can it be assumed that published performance metrics have been achieved. The alternative is not good practice, and can induce a false sense of security, compounding error and lack of

traceability with the additional nefarious effect of encouraging less rigorous IQC studies by stakeholder laboratories [ROC 11].

A comprehensive marine nutrient monitoring program requires that nutrients be monitored under a vast range of different aqueous samples, hence with due consideration of analytical matrix effects, in particular salinity interferences in marine nutrient analyses, especially if CFA is employed (e.g. [MAN 83, STE 96, AMI 97, WOO 10]). This so-called “salt-effect” is due to diffraction effects in the colorimeters that are detected as absorbance signals, thus interfering with the nutrient signal [KIR 96]. This salinity effect must be measured and corrected for in calculating the final results [AMI 07, AMI 09]; it does not necessarily lead to a poor method performance, but the operator needs to be aware of these factors and proceed with an appropriate adaptation of the operational conditions of the CFA system in use [ROC 11], and a suitable verification of the accuracy and precision criteria for method performance [IBE 95].

4.4.3.2. Laboratory performance studies

The awareness about the lack of comparability and traceability for seawater nutrient analysis worldwide (and generally of lack of validation of data) was conducted by ICES and UNESCO to perform a number of laboratory performance studies [UNE 67, KIR 91], which were followed up in Europe by the Quality Assurance of Information for Marine Environmental Monitoring (QUASIMEME) programs in the early 1990s [WEL 93, COF 94]. For nutrients, the assessment of data quality indicated that in general, systematic rather than random errors were the major cause of poor analytical performance, hence justifying the need to efficiently adapt the analytical range of the instrumentation to the matrix and analyte concentrations in the samples [AMI 97]. One of the strong points made by the QUASIMEME program was that, irrespective of the method employed in the determination of nutrients, basic IQC procedures and analytical chemistry principles remained the central foundation upon which metrological quality lies [ROC 11], i.e. analytical systems have to be adjusted to the appropriate range of concentrations and systematic control of the analytical sensitivity, assessment and

correction for the blank and calibration procedures with appropriate care for any matrix effects has to be undertaken.

Let us consider that QUASIMEME and similar performance studies generally consider only laboratory analyses, and not the overall analytical chain, i.e. sampling and sample handling as well as preservation and storage methods are rarely assessed. This upstream part of the analytical chain has been addressed by another EU-funded program called “Quality Assurance for Sample Handling” (QUASH). Findings showed that, from the point of view of improving analytical accuracy, determination of nutrients in seawater samples should be carried out immediately following collection, thus avoiding any intermediate preservation or storage; once any increase in sample handling or any manipulation occurs, then that will necessarily amplify the risk of random errors [ROC 11]. Another reason for avoiding the deferral of nutrient analysis after sample collection is the possibility of sample alteration during storage and handling, since nutrients are unstable in seawater and their *in vitro* reactivity is high, in particular NH_4^+ , NO_2^- and PO_4^{3-} . These substances are also prone to high contamination risks during manipulation, and standard operational procedures should always account for potential contamination sources [KER 87].

Dore *et al.* [DOR 96] reviewed preservation methods in the context of quality assurance of quantitative nutrient analysis in the period 1953–1995. The study convincingly shows that frozen storage (minimum -20°C) of unfiltered open ocean waters in clear polyethylene bottles reasonably preserves, in decreasing order of efficiency, the *in situ* concentrations of nitrate + nitrite, silicate and phosphate. In addition, samples for the determination of silicate should be allowed to thaw for a minimum of 12–24 hr or more to allow depolymerization to occur prior to analysis [MAC 86]. The addition of chemical preservatives, of which mercuric chloride [KIR 92, KAT 99] is the most effective, presents other issues as well, since it systematically lowers the ammonium concentrations in stored samples by ~10 % [KAT 99], and can poison the nitrate channel’s copper-cadmium reduction column, hence affecting its reducing capacity [ROC 11].

4.4.3.3. *The International Nutrients Scale System*

It has been well established that despite continuing efforts, adequate comparability and traceability of marine nutrient data worldwide has not been achieved so far. However, suitable reference materials should ideally be available in large quantities and besides being prepared in a natural seawater matrix, they should also have the potential to achieve comparability of nutrient analysis to a quantifiably high level [ROC 11]. In combination with good analytical laboratory practice and the use of tracking standards through measurement campaigns, these should be a crucial part in accomplishing the level of traceability necessary to reconcile different datasets collected globally. Following lessons learnt from performance studies [AMI 97, AOY 10], a collaborative program called the International Nutrient Scale System (INSS) was launched in Japan, aiming to establish global comparability and traceability of nutrient data [AOY 10]. A joint ICES-IOC study group on nutrient standards (SGONS) was created in 2009 with the aim of developing international standards for nutrients to establish comparability and traceability of nutrient data in the world oceans [ICE 09]. These international efforts clearly demonstrate the need to develop a common, certified reference seawater standard for dissolved nutrients, which would allow (1) traceability to be achieved within the full analytical chain, which subsequently would permit (2) day-to-day quality control procedures to be established and on this basis, performance improved and validated on a continuous basis [ROC 11]. These traceability needs are discussed in the concluding chapter of this book.

Conclusions: Achieving Traceability in Marine Monitoring Measurements?

5.1. Metrology in marine chemistry: traceability principles of chemical measurements

Sound strategies for marine chemical monitoring call for measurement systems capable of producing data of demonstrated quality. Monitoring the quality of the marine environment principally relies on the analysis of abiotic matrices such as water, suspended matter and sediment, as well as biological matrices (e.g. algal and biological tissues), for a wide variety of toxic and carcinogenic substances at various levels of concentrations. This issue is becoming increasingly complex regarding the analytical problems encountered and the pressure that laboratories are facing with respect to providing fit-for-purpose data quality. The last decade has seen an increasing awareness for QA of environmental analysis, which has been reflected by the development of a number of guidelines and documented standards, e.g. for managerial aspects and technical operations (e.g. sampling and method validation), and tools, e.g. reference materials and proficiency testing schemes [QUE 95, SUB 95, GÜN 96, QUE 99c, BAR 00].

Traceability of data is not a new concept as it has been debated since the early 2000s. This concept is a heritage of metrology as

conceived for physical measurements (e.g. mass, length, time and temperature) more than one century ago. Metrology in chemistry and biology is now actively discussed among experts in metrology, analytical chemistry and biology in order to propose a system that would be applicable to complex chemical and biological measurements [VAL 98, KIN 99], and the application of metrology concepts to environmental analysis has also been examined [QUE 01, QUE 04]. The discussions generally point out that the direct application of theoretical metrology concepts to chemical and biological measurements is not possible because of major differences between chemical/biological and physical measurement processes. For example, chemical analysis results are often strongly dependent upon the nature of samples (whereas physical measurements are less or not affected). A wide variety of analytical problems are encountered in relation to different parameters and matrices (preventing standardized general procedures to be used for all cases), preliminary steps are necessary (e.g. sampling and sample pretreatment) that may have an effect on the final result and so forth.

When dealing with environmental monitoring in general (and marine monitoring in particular), these theoretical discussions seem to be very distant from real-life situations, and the practice is, in most cases, very far from the theory. Even though the situation has drastically improved within the last few years, the warning made at the beginning of the 1990s [GRI 90, HOR 92] is still relevant: many (marine) environmental chemists still do not pay sufficient attention to the reliability of analytical results and confuse trueness and precision. With respect to traceability, the situation is even worse and this concept is prone to many misunderstandings when it is applied to marine chemical measurements. This chapter is based on previous publications of the author [QUE 01, QUE 04] about chemical metrology with the focus here on traceability in the context of marine chemical monitoring.

5.1.1. *Meaning of traceability for chemical measurements*

ISO defines traceability as “the property of the result of a measurement or the value of a standard whereby it can be related to

stated references, usually national or international standards, through an unbroken chain of comparisons all having stated uncertainties” [ISO 93]. In this definition, three key elements may be distinguished, which have been extensively discussed with respect to their applicability to chemical measurements: (1) the link to stated references, (2) the unbroken chain of comparisons and (3) the stated uncertainties. Detailed discussions have already investigated how these elements apply to chemical measurements [VAL 99, WAL 99, QUE 00]; let us examine now how they may be understood in the context of chemical marine monitoring.

In the definition, the *stated references* may be reference methods, reference materials or SI units (kilogram or mole for chemical measurements) [KIN 97]. In theory, all chemical measurements should aim at being traceable to SI units. In practice, measurements correspond to approximations via comparisons of amounts and instrumental response generated by a number of particles and so forth, and establishing SI traceability nowadays demonstrates to what extent these approximations are clearly related to the stated references [VAL 98, VAL 99]. As discussed below, most of the chemical measurements performed in the context of environmental monitoring are actually traceable to either a reference material (pure substances or matrix CRMs) or to a reference method (e.g. standardized method).

The *unbroken chain of comparison* basically means that there is no loss of information during the analytical procedure (e.g. incomplete recovery and contamination). Achieving this condition is difficult to varying degrees according to the analytical problem considered. It will be more critical for techniques involving successive analytical steps (e.g. extraction, separation and detection) and less acute for direct measurement procedures (e.g. sensors) that may, however, be faced with other difficulties (e.g. lack of sensitivity or selectivity). Marine chemical monitoring causes an additional difficulty, i.e. sample collection and storage. These two steps form an integral part of the traceability chain, which is too often forgotten.

The third key element, the *stated uncertainties*, is also a critical feature that many analysts still overlook. The theory implies that the

uncertainty of a measurement is based on the traceability and the uncertainty of all the stated references that contribute to this measurement. In other words, uncertainty components should be estimated at each step of an analytical process, i.e. the smaller the chain of comparison the better the uncertainty of the final result. Here again theory is confronted with practice when dealing with complex measurements such as those performed in the framework of marine monitoring. This chapter will not discuss uncertainty matters, which are widely discussed in the literature [GİN 96, VAL 98, MAR 99]. Discussions will instead focus on the first two elements, which are more likely to be prone to misunderstandings.

Before starting these discussions, it is useful to remind the reader that traceability should not be confused with accuracy. The latter covers the terms *trueness* (closeness of agreement between the “true value” and the measured value) and *precision* (closeness of agreement between the results obtained by applying the same experimental procedure several times under prescribed conditions). General aspects of QA in long-term environmental monitoring (including considerations on accuracy) have been extensively discussed in the literature [QUE 95, QUE 99b, QUE 99c] and will not be repeated here. Let us highlight the fact that a method that is traceable to a given stated reference is not necessarily accurate (i.e. the stated reference is not necessarily corresponding to the “true value”), whereas an accurate method is always traceable to what is considered to be the best approximation of the true value (defined as “a value, which would be obtained by measurement, if the quantity could be completely defined and if all measurement imperfections could be eliminated”) [ISO 93]. At another level, precision and uncertainty are also often confused and considered to be similar concepts, which is not correct since the uncertainty includes both random and systematic errors (while precision is solely linked to random errors) [VAL 99].

Measurements performed for marine chemical monitoring are based on a succession of actions, namely (1) sampling, storage and preservation of representative samples, (2) pretreatment of a sample portion for quantitation, (3) calibration, (4) final determination and

(5) calculation and presentation of results. Considering this, we may now consider in further detail what the types of stated references used in marine monitoring are.

5.1.2. Stated references

5.1.2.1. SI units

Units of the “International System” (SI) correspond to internationally recognized fundamental units that are used in metrology. They establish units of length (m), mass (kg), time (s), temperature (Kelvin), etc. The unit that underpins chemical measurements is the unit of amount of substance (the mole). In principle, all chemical measurement data should be traceable to the mole [KIN 99]. In practice, contrarily, e.g. to the mass standard, there is no “ ^{12}C mole” standard, and the kilogram is needed to define the mole [ISO 93]. Therefore, chemical measurements are actually traceable to the mass unit (the kilogram). Marine chemical measurements are based on the determination of the amount of substance per mass of matrix. One should not confuse this traceability to mass units with the traceability to the “true value” of the substance in the matrix. This is discussed in the following.

5.1.2.2. Documented standards

Standardization is an important aspect of routine environmental (marine) monitoring. Documented standards (norms) related to measurement procedures are designed to establish minimum quality requirements and to improve the comparability of analytical results. They also often represent the first step of the introduction of techniques/methods into regulations. In this case, the reference is closely related to the documented protocol, representing one of the main links of the traceability chain. This aspect will be particularly acute when dealing with operationally defined parameters, i.e. parameters determined following a strict analytical protocol [URE 95, QUE 02] since the traceability chain may be broken if the protocol is not strictly followed.

Standardized procedures (documented standards) have been developed for sampling strategies and analytical techniques (documented protocols describing in details analytical procedures, from the subsampling to the actual determination). In the area of environmental monitoring, the implementation of documented standards bound to regulations has been criticized (e.g. EPA methods) since standardized methods may become outdated while still being enforced by regulations that have not been revised. It may indeed happen that the analytical state of the art in a certain field has drastically improved but laboratories are still obliged to use old-fashioned inferior methods for legal reasons. Standardization bodies have recognized this problem and now allow the progress in analytical technologies to flow into standardization work. The use of a standardized method does not guarantee that no errors will occur; they only provide an analytical framework that is considered as the reference for a given measurement.

Let us consider a specific example: the determination of extractable forms of elements using a single or sequential extraction procedure. In the marine field, this approach is regularly used for sediment analysis for studies of mobility of trace element [QUE 98]. The measurements do not relate to specific forms of elements but rather to amounts extracted by a given procedure and operationally defined according to interpretations such as “mobile” and “carbonate-bound” forms. The comparability of data is only possible if the extraction (documented) protocols are strictly followed, i.e. the traceability of the final results will be linked to the documented extraction protocol taken as reference. If a change in operational parameters is made by a laboratory, the traceability link will be broken. This comment applies to all the measurements that correspond to a partial extraction of a substance in a given medium.

Detailed (documented) guidelines are difficult to set up for sample collection and storage, which however remain the primary source of error in environmental monitoring (and hence one of the weakest links of the traceability chain). Recommendations are available in the scientific literature [QUE 95, BAR 00], but there are very few

examples of documented standards that can formally be used as stated references in the framework of marine monitoring. Sampling standards generally define the method of sampling, the number of samples to be collected, their representation, the frequency of sampling (taking into account natural variations), the sampling techniques and tools, etc. Statistical sampling tools exist [GAR 91, GY 91], but they are often neglected and hardly applicable to practical cases. The nature of the sample and the substance to be monitored actually dictate the choice of the sampling, which is hence adapted case by case. A similar situation is encountered for sample storage for which recommendations are given with respect to protection of the samples from light and elevated temperatures. This situation is obviously unsatisfactory with respect to the comparability of data since no clear stated references may be presently used.

5.1.2.3. *Reference methods*

Analytical methods differ in the link between the signal produced by a given determined substance and the signal obtained from the calibration material. For the vast majority of methods used in marine monitoring, the link is usually related to an amount of substance of established purity and stoichiometry. In some fields, e.g. the determination of chemical forms of elements (also referred to as “speciation” [COR01], the techniques are based on a succession of analytical steps such as extraction, derivatization, separation and detection. This multiplies the risk that the traceability chain is broken owing to a lack of proper tools (e.g. reference materials containing actual analyzed species and secondary standards) to accurately determine the result, e.g. extraction recoveries and derivatization yields [QUE 00]. For other methods, e.g. X-Ray Fluorescence (XRF), the link is through CRMs.

The so-called primary methods are methods with the highest metrological qualities, for which the uncertainty can be established in terms of SI units and for which the result is accepted without reference to an external calibrating material. These methods have few random errors and are supposed to be exempt of systematic errors; they are also referred to as “definitive”, “absolute” or “stoichiometric”

methods (e.g. gravimetry, titrimetry and coulometry of simple solutions) [VAL 98]. Using primary methods guarantees, in principle, that measurements will be traceable to SI units, i.e. traceability links will be established to the “true value” of amount of substance. We would think that “reference methods” should hence systematically be “definitive methods”. However, these methods mainly exist for trace element determinations. For organic or organometallic compounds, there are no real definitive (or primary) methods for the reasons mentioned above (analytical steps with impossibility to firmly demonstrate full recovery).

As it has been stated earlier, primary methods theoretically enable the traceability of chemical measurements to the SI unit (i.e. to the mole) to be achieved. This has been demonstrated for relatively “simple” measurements, such as trace elements in seawater, using IDMS [DE, 96]. However, what can be obtained for inorganic parameters in water samples is far from achievable for the analysis of complex organic substances and matrices requiring a series of analytical steps (e.g. extraction and clean-up). In this case, the traceability chain will be broken at several stages and the stated references will only rely on approximations (recovery estimates). The better these approximations, the closer the traceability of the measurement to the true value. In many cases (e.g. for trace organic and organometallic determinations), true “definitive” methods do not exist for marine chemical measurements since there are no means at present to give proof that extraction or chemical reactions (e.g. derivatization) have yielded a 100% recovery. As an example, ID-ICPMS has been used for determining TBT in sediment and mussel matrices after HPLC separation [RIV 01]: we could think that the measurements then demonstrated trueness since, in principle, no loss could occur after separation. The situation was, however, that the final results were traceable to the “true” value of TBT present in the extract but not necessarily to the true value in the sample; the link to extraction recovery simply hampers this traceability being achieved.

It has been argued that the development of “reference measurement” procedures that would be adequately applicable to real

sample matrices (rather than matrix-dependent methods) would be a much better trend than trying to develop thousands of matrix-matched CRMs (see section 2.3.4) [DE 96]. However, these “reference methods” also need to be validated, which cannot negate the necessity to develop suitable CRMs.

Methods based on internal or external calibration rely on the availability of calibrants of high purity and verified stoichiometry but this is only the last link of the traceability chain (i.e. calibration of the detector signal). In principle, all the steps of an analytical technique should be recorded in such a way that the result of the final determination is linked through an unbroken chain of comparisons to appropriate standards. In other words, firmly establishing traceability in analytical measurements means that several “primary” chemical reference materials in the form of (ultra) pure substances are interlinked by well-known, quantitative, high-precision high-accuracy chemical reactions [DE 96]. In practice, this is not achievable for the vast category of (marine) environmental measurements, and there are many “weak links” in the traceability chain for a wide range of analytical measurements. Starting from extraction, there is no way at present to firmly ensure that a substance has been fully recovered from a complex matrix; methods that are generally used (e.g. successive extractions or spiking procedures) enable the estimate of the method reproducibility but do not necessarily demonstrate full recovery. For methods including a derivatization step, there are few or no appropriate calibrants available to date to check the yield of derivatization reactions, which represent an additional gap in the traceability chain. As a conclusion, these methods are dependent on a number of more or less well-controlled parameters that may vary from one sample to another. As stated below, the analytical steps that rely on a recovery estimate can only be validated in comparison to independent methods, giving a good indication on data comparability but not necessarily on accuracy. Hence, few of these methods may be considered as reference methods unless they are documented with a great level of detail, describing all the analytical operations and the limits of applicability of the method. This is the case of “official methods” that required regulations. These are faced with the problem

discussed above regarding documented standards (possible risks of becoming outdated).

5.1.2.4. *Reference materials*

In principle, the role of reference materials is well known. CRMs may be calibration materials (pure substances or solutions, or materials of known composition for techniques requiring matrix-matched calibrants, e.g. XRF) or matrix materials representing as far as possible “real matrices” for the verification of measurement process. LRMs (also known as QC materials) have the same basic requirements of representativeness, homogeneity and stability, but these materials are not certified and generally produced at a much smaller scale, e.g. for interlaboratory studies or internal QC (control charts), i.e. to monitor the performance of analytical methods with time (reproducibility) through the establishment of control charts [HAR 90]. In this view, control charts and related RMs may be considered as long-term stated references for analytical measurements. It has been stated that the “reference” represented by a RM may not always be reliable since, in many cases, the RM does not have the “same” matrix as the unknown sample [DE 96]. This is discussed in the following.

Official organizations attempt, wherever possible, to produce reference materials estimating the true values as closely as possible. In the case of matrix (marine) environmental CRMs, this is mainly achieved by employing a variety of methods with different measurement principles in the material certification study; if these methods are in good agreement, one may assume (but not firmly demonstrate) that no systematic error has been left undetected, and the reference (certified) values are the closest estimate of the true value. This approach possibly includes definitive methods (see above), which seldom exist for analyses involving an extraction or derivatization step. In many instances, *consensus* values are accepted as *true* values reflecting the state of the art (hence ensuring data comparability). Discussions are on-going on the fact that many matrix CRMs do not guarantee a full verification of accuracy owing to

possible remaining bias (e.g. all extraction methodologies, although being in good agreement, could be biased to a certain degree, with no means to demonstrate it at the present stage). This is a point of discussion in the following.

As mentioned earlier, some (certified) RMs are intended for calibration purposes; in these cases, the uncertainty of the certified value is of prime importance since it will affect the final uncertainty of the measured value in the unknown sample. In the case of certified pure substances or calibrating solutions, the uncertainties of certified values are usually negligible in comparison with the method uncertainty. This is not the case of matrix CRMs that are in principle reserved to the validation of methods; these materials are used for calibration purposes for non-destructive methods (e.g. XRF) and the larger uncertainty of the certified values may lead to a too large uncertainty of the final results (thus leading to semi-quantitative measurements).

In the field of marine chemical monitoring, the wide variety of matrices and substances encountered calls for a large availability of matrix reference materials representative from various sample types (e.g. sediments, suspended matters, waters and biological tissues), each of them displaying a wide range of sample compositions. Reference materials represent “physical” stated references to which measurements can be linked. As mentioned above, this traceability is often criticized since the requirement of matrix similarity between unknown samples and matrix CRMs is never achievable in practice, and compromises often have to be found. It should be stated that a correct result obtained with a matrix CRM does not give a full assurance that “correct results” will be achieved when analyzing unknown samples, owing to differences in matrix composition [QUE 00].

The question of traceability of matrix CRMs (representing complex chemical systems) to SI units, and hence their values as “reference”, has been an on-going debate since the early 2000s. Traceability implies “an accurate realization of the unit in which the property

values are expressed". Similarly to the achievement of traceability to SI units, the "accurate realization" is often hardly demonstrated in practice. Indeed, as discussed in section 3.3, it is difficult to demonstrate that a 100% extraction recovery has been obtained for a given substance in a complex environmental matrix. The assumption of a 100% recovery will actually be more valid if the certified values have been obtained in the frame of interlaboratory studies using a variety of (different) techniques. Even though, in the absence of "primary" (or "definitive") methods, the collaboratively obtained value may only be considered as "consensus value" reflecting the stateoftheart of a given method. This consensus value represents an excellent reference to achieve traceability in a given area, but does not necessarily correspond to the "true value" (which is actually not quantifiable in most of complex environmental measurements).

In addition, there are numerous fields of marine chemical monitoring for which RMs are lacking (or are available but with a matrix too far removed from the analyzed samples) or cannot be prepared owing to their instability. This hampers traceability being achieved. In this case, other approaches have to be followed (e.g. interlaboratory studies). In case of good correspondence between the matrix of samples and the matrix of CRMs, this reference is certainly the most appropriate one to check the accuracy of analytical methods, and compare the performance of a method with other methods (or other laboratories). Similar comments with respect to representativeness may be made concerning matrix LRMs used for internal QC purposes (establishment of control charts).

In order to clarify the traceability links represented by reference materials, a classification has been established, categorizing the various types of materials [PAN 97] as shown in Table 5.1. In this classification, primary RMs are traceable to SI units through primary methods and physical standards (i.e. mass standards); CRMs and LRMs certified or reference values are obtained by reference or validated methods, but they are not mentioned to be necessarily traceable to SI units. The table presents a traceability link between LRMs, CRMs and primary RMs, which may be achieved in some cases but not as a general rule, in particular for matrix RMs as

discussed earlier. In other words, even if primary RMs are used for calibrating an analytical method used for obtaining certified (or reference) values, the uncertainties that may remain on recoveries for example do not allow us to firmly establish traceability to the primary RMs (and hence to SI units). This is discussed in one of the case studies below.

Level	Appellation	Criteria
I	Primary RM	<ul style="list-style-type: none"> – Materials with the highest metrological qualities, of which the values are determined (certified) by a primary method – Developed by a national metrological institute – Recognized by national decision – Traceable to SI units and verified by international intercomparisons
II	Certified RM	<ul style="list-style-type: none"> – Fulfill the ISO Guide 30 definition – Generally developed by a national reference laboratory or a specialized organization – Certified by reference methods, by comparisons of different methods or a combination of the two approaches – Recognized by national or specialized organizations – Accompanied by a certificate indicating the uncertainty of the certified values and describing the traceability
III	Working RM (or laboratory RM, or quality control material)	<ul style="list-style-type: none"> – Fulfill the ISO Guide 30 definition – Produced by an accredited organization – Establishment of reference values by one or more validated methods – Accompanied by a description of the achieved traceability and giving an estimate of the uncertainty

Table 5.1. Classification of chemical reference materials (adapted from [PAN 97])

5.1.2.5. *Environmental specimens*

Specimen banking is another type of stated references that may be used in environmental monitoring in general and marine monitoring in particular. The approach consists of collecting samples, processing them and storing them on a long-term basis under conditions that prevent any significant changes in their chemical composition [EMO 97]. The aim is to create a systematic repository of environmental samples, providing information about current levels of pollution and tools to evaluate contamination trends. This approach certainly represents the best referential system for long-term environmental monitoring. Besides the specimens that are “true” stated references of the environment status at the time, reference materials can also be produced from surplus specimen material (i.e. stabilizing them by freeze-drying) in order to monitor the reproducibility of analytical techniques, hence ensuring internal QC using the most representative samples [EMO 97]. “Fresh” reference materials may also be prepared from samples similar to collected specimens and processed the same way (i.e. in an uninterrupted cryochain to preserve their integrity), homogenized and stored as fresh powder materials for the purpose of developing new analytical procedures, optimizing existing methods, internal QC and stability experiments of environmental specimens [SON 97].

A sound, continuous, follow-up of the trends of marine environmental quality can only be based on a well-structured monitoring system based on the selection of reference sites and the regular monitoring of selected substances and matrices. As described earlier, such a system relies on the long-term storage of environmental specimens that are stored under optimal conditions (in liquid nitrogen) and that serve the purpose of monitoring temporal and spatial contamination trends. The specimens, taken as stated references, would also enable to repeat analyses focusing on specific contamination studies when more sophisticated (accurate) analytical methods would become available. Such an approach has been implemented at the national scale in some countries (e.g. Germany and United States). This is certainly the best system for a long-term monitoring strategy of the environment quality, which could be extended internationally. The difficulty would then be related to the possible lack of a harmonized

approach across countries, i.e. each country following its own strategy would hamper a good worldwide comparability of data to be achieved. This approach is discussed in section 5.1.3.

5.1.2.6. *Proficiency testing*

Participating in interlaboratory studies (or proficiency testing, which is the equivalent term used in regulations) is a way for laboratories to establish stated references for evaluating the performance of their methods. These exercises imply that one or more materials are distributed to several laboratories for the determination of given substances. The comparison of different methods enables the detection of possible sources of errors linked to a specific procedure or the way a method is applied by a given laboratory. Exercises focusing on a single method enable the establishment of performance criteria (e.g. precision).

The stated references, here again, may be reference materials that should meet homogeneity and stability requirements. However, contrarily to reference materials used for internal QC, proficiency testing may involve samples with a limited shelf life that are distributed to laboratories for analysis of particular parameters that could not be evaluated using stabilized RMs. Examples are “fresh” materials, e.g. biological tissues, with a short-term preservation period.

Proficiency testing may also focus on “stated references” that are either reference sites, e.g. a well-characterized site for the evaluation of sampling procedures, or a bulk common sample to be analyzed by the laboratories at the same time, e.g. a tank full of seawater for microbiological measurements.

Similarly to what has been discussed for reference materials, the measurement values obtained in relation to interlaboratory studies (using different techniques) are taken as the “best representation of the state of the art”, i.e. offering an excellent mean for laboratories to achieve comparability (i.e. traceability) of their results to a recognized reference, which is in this case a consensus value (generally the mean of laboratory means). This reference does not enable traceability to the true value of the substance in the medium to be achieved, but it

represents a very useful method for achieving comparability of environmental measurements.

5.1.3. Case studies illustrating metrology in marine chemistry

5.1.3.1. Approaching the closeness of traceability to SI: trace elements in seawater

The first example corresponds to the most “simple” system that may enable the traceability of marine chemical measurements to the SI unit to be achieved, i.e. trace element monitoring in seawater (Figure 5.1). Traceability of trace element determinations in seawater to the mole using IDMS has been claimed as studied in the framework of collaborative studies among metrological institutes [VAN 96]. Let us examine whether this traceability could be maintained in the framework of a monitoring exercise, even using IDMS as a final detection method.

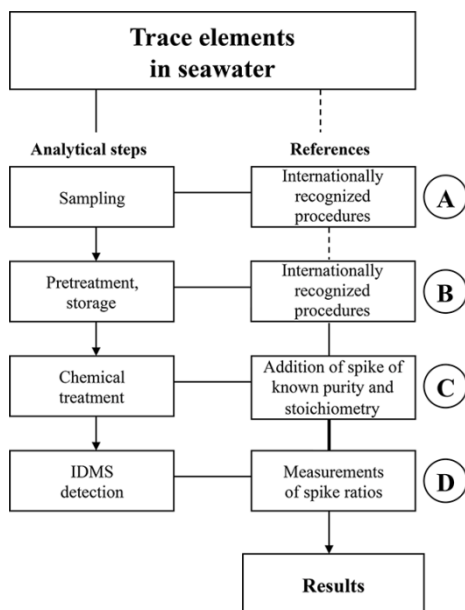


Figure 5.1. Traceability chain for trace element determinations in seawater (adapted from [QUE 99b])

Figure 5.1 distinguishes four steps, separated into an operational part (sampling to detection) and a reference part representing the traceability to stated references.

– Step A: sampling is recognized to represent the major contribution to analytical errors. A summary of trace element data in oceanic waters over 20 years (1965–1986) had shown that the concentrations were regularly decreasing, which was against all other findings regarding increased environment contamination levels [TOP86]. As an example, copper decreased from 3.0 to 0.25 $\mu\text{g L}^{-1}$, lead from 0.03 to 0.002 $\mu\text{g L}^{-1}$, zinc from 10.0 to 0.39 $\mu\text{g L}^{-1}$, etc. Considering that the trace element concentrations in open seawaters are recognized to be relatively constant, these huge differences were only due to contamination during sampling and sample storage. No serious conclusions on contamination trends over 20 years could hence be made. Since the beginning of the 1980s, considerable improvements have been made with respect to sample collection and storage, of which recommendations have been published in the literature and followed in the framework of oceanographic monitoring campaigns [SCA 82, BER 83] and certification of seawater reference materials [BER 83, QUE 92]. All precautions are taken to minimize contamination, carefully selecting the sampling materials (e.g. polyethylene, Teflon and silicone tubing) and following strict guidelines for the cleaning of containers. The reference here relies on the experience gained by oceanographers and documented guidelines that have been adopted as a consensus. It is not possible to demonstrate that all contamination sources have been avoided (explaining the dotted line in the reference links), but this is the best consensus that may be achieved for seawater analysis. A way to control the likelihood of contamination is to process a blank sample using the same procedures and materials as used for the unknown sample.

– Step B: similarly to sampling, sample pretreatment and storage represent a high risk of contamination and procedures were tested to minimize these sources of errors [BER 83]. Seawater samples are generally acidified on board (to a pH value below 1.6) in order to stabilize them and stored at ambient temperature. Here again, we have to rely on the common experience to assume that the traceability has

not been drastically affected at this stage. Errors can be due to the addition of acids of insufficient purity, errors of manipulation, etc. As observed in step A, the reference link is also weak in this case, since it is hardly possible to firmly identify all errors that may occur on board.

– Step C: this step corresponds to the laboratory work, in this case, determination of the trace elements by IDMS. The method is considered to be a primary method as it is based on the addition of a known amount (determined by weighing) of the analyte in an isotopic composition different from that of the analyte present in the sample. The spiking is carried out prior to the chemical treatment of the sample and has to be performed in such a way that an equilibrium is reached between the spike and the isotopes naturally present in the sample. Considering the similarity between the isotopes, the chemical treatment does not affect the isotopic ratio even if the analyte recovery is not complete. Possible contamination or losses have no effects on the result traceability since the analyte and the spike will undergo the same pattern and the ratio will not be affected.

– Step D: the amount of analyte is related to the amount of the spiked isotope according to a known formula [RIC 97]. The calculation is only based on isotopic ratios that may accurately be measured by mass spectrometry. The measurement is hence considered to be traceable to the mole.

In the context of trace element monitoring in seawater using IDMS as a determination technique, the weakest parts of the chain are hence the sample collection and pretreatment steps. An improvement of the traceability chain could be achieved if the isotope spiking was to be carried out immediately after the collection, i.e. at the pretreatment stage (step B). IDMS measurements, in theory, guarantee the traceability of the pretreated sample to the SI unit. However, the application of this technique in interlaboratory studies on trace elements in seawater have shown that even if this method is considered to be a primary method, it is not without errors owing to operating difficulties; an example has been published, showing that five laboratories out of 16 using IDMS reached the required performance for the certification of seawater CRMs [KIN 97]. As a conclusion, we may realize that even a relatively “simple” system

with respect to the analytical measurements is not exempt from sources of errors, either due to possible contamination occurring, e.g. at the sampling stage (with few means to firmly demonstrate the lack of contamination, in particular for the sampling itself) or manipulation errors at the determination stage (due to the operating complexity of IDMS).

5.1.3.2. A more common situation with respect to traceability in marine monitoring: organotin compounds in harbor dredges

In this case study, the substance of concern is more complex (organotin compounds, more specifically TBT, issued from release of antifouling paints) and requires a more sophisticated measurement approach (Figure 5.2).

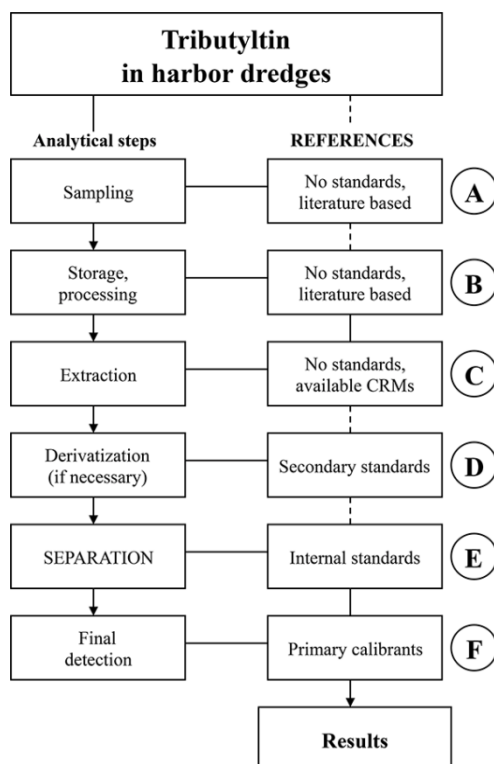


Figure 5.2. Traceability chain for tributyltin determinations in harbor dredges (adapted from [QUE 99b])

TBT is prone to possible degradation if insufficient care is taken at the sample collection and storage steps. The matrix of concern (harbor dredge) is also more complex, constituted by a mixture of mineral particles of various densities and organic particles (various debris of mollusc shells, algae, etc.). Analytical procedures have been extensively reviewed for organotin compounds [DE 96, QUE 96, DON 01], highlighting that the measurement traceability chain presents many weak links in comparison to the first case study.

– Steps A and B: contrarily to the first case study, there is no formal recommendations regarding sample collection (step A) and storage (step B) for the monitoring of TBT in harbor dredges. A laboratory will generally rely on published procedures, which are often described along general lines, lacking details on QA. Procedures will hence be adapted case by case, following “homemade” approaches, which are often hardly comparable *sensu stricto* from one laboratory to another and do not allow to firmly establish traceability owing to the lack of well-defined stated references. In other words, the data produced on the basis of “in-house made recipes” may be, or not, of good quality. There is simply no means to anchor them on a firm reference system.

– Step C: extraction methodologies also vary considerably from one laboratory to another. The extraction recoveries are in principle calculated, but here also, despite discussions in the early 2000s, there is still no real consensus on the approach to be followed [QUE 00]. We may say that there is no real need to set up documented extraction protocols that could rapidly become outdated with the constantly improving analytical methodologies, and that it is only necessary to demonstrate that the methods used are validated. This validation is indeed possible on the basis of existing CRMs with a matrix representative of harbor sediments [QUE 00]. The recovery check is, however, prone to the uncertainties discussed in Chapter 2 with respect to the “similarity” of matrix composition between the CRM and the unknown. Therefore, the traceability link exists but it is still questionable.

– Step D: derivatization reactions (e.g. hydride generation and Grignard reactions) are frequently used for the determination of TBT in environmental matrices [MOR 00]. The traceability chain implies,

in principle, that derivatization yields are verified, which is only possible on the basis of available “secondary” standards, i.e. pure derivatized TBT calibrants (e.g. ethylated and pentylated). This validation has been followed in the early 2000s in the case of certification of reference materials [QUE 00], but it did not turn into a routine practice. As in step C, the completeness of the derivatization yield will be evaluated on the basis of analysis of matrix CRMs, with the already expressed limitations.

– Step E: separation will be necessary to isolate the different organotin compounds from possible interfering compounds from the matrix. Selectivity is the key feature here. The risk of losing traceability is related to a possible degradation of TBT (heat-induced degradation on the column) or an insufficient selectivity. Internal standards with a composition close to TBT are useful stated references to detect possible losses or insufficient species separation. This part of the traceability chain is considered to be reasonably under control.

– Step F: high-purity primary standards for the calibration of TBT measurements are available and this final link of the traceability chain (detection) is considered to be satisfactory.

This case study shows that a “real case” monitoring exercise is subject to many questions with respect to measurement traceability. The weakest links are certainly the sample collection and storage for which no strong reference system exists. The situation is better with regard to the analytical measurements, even if the links are not considered to be that strong (e.g. with respect to evaluation of analyte recoveries). At present, we must admit that we are far from being able to firmly demonstrate measurement traceability to the “true value” of TBT in the environment.

5.1.3.3. A holistic metrological system for marine chemical monitoring?

There is no single “perfect” measurement system that would suit all the needs for marine chemical monitoring, considering the wide variety of situations and the on-going progress of analytical technologies. As a preamble, we should note that the multiplicity of studies throughout the world and the huge production of data still do

not necessarily enable a sound (accurate) estimate of the global quality of our marine environment to be performed. The main reason is the lack of a proper referential system that would allow a large-scale follow-up of possible contamination trends both temporally and spatially. Note that many studies carried out are of questionable quality; many of them fulfill their aim at the regional or national level, with their own reference system that allows conclusions and decisions to be taken with respect to marine status. The problem is, however, when an attempt is made to compare data produced by different organizations, i.e. across borders. Many situations have shown that, owing to a lack of common stated references, the monitoring data were not comparable and all efforts to establish contamination mapping or modeling often failed for this reason. Figure 5.3 describes a possible referential system for a holistic approach of marine quality monitoring, in particular for solid matrices (the system may be applied to water with slight modifications with respect to the preparation/storage of reference materials).

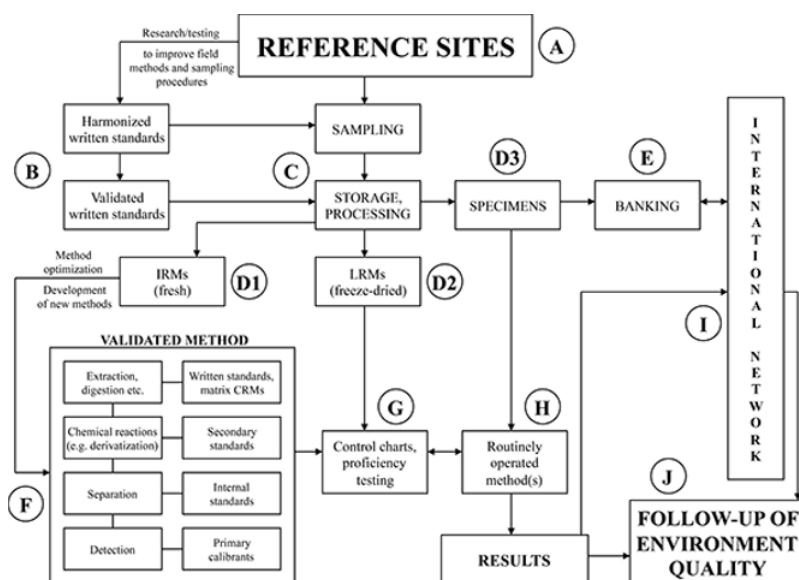


Figure 5.3. Holistic approach for achieving traceability of marine monitoring measurements (adapted from [QUE 99b])

The concept of reference sites is not new and environment quality is monitored at regional or national levels in some countries (e.g. Germany and United States) using this approach. Let us examine this system step by step to discuss the strength of the traceability chain in such a holistic referential approach:

- Step A: the starting point is a selection of reference sites representative of various marine environments (continental to off-shore) that are chosen according to a series of criteria such as homogeneity, contamination sources and easiness of sample collection. This reference is actually the basic traceability link for a sound follow-up of environment quality (last step of the system, J).

- Step B: the selection of sites enables to perform research/testing on field procedures (sampling, field measurement methods), which allows to harmonize sampling procedures and validate storage procedures. Documented standards (SOPs) are necessary to ensure comparability of data at the international level. These standards have to be designed, tested and approved in the framework of an international network (step I in the figure) that provides regular data on environmental status. An important note is that these standards may be improved by on-going research/testing activities on the site, and that the stated references may hence evolve along the progress in analytical sciences. In case of improvement of methodologies and amendment of the standards, a traceability link has to be established between data produced within the “old” referential system and the new one, and all parties have to be informed about the changes. This way, all the monitoring data are preserved and the trend studies may continue on improved technological bases.

- Step C: this consists of operational procedures, which are closely linked to the documented standards (step B). The strength of the link depends on how well the procedures will adhere to the documented standards used as common stated references.

- Step D: this mentions “physical stated references”, i.e. reference materials representative of the monitored reference sites. They should be produced from the samples collected at regular intervals within the monitoring framework. Three categories of stated references are defined. Internal reference materials (IRMs), step D1, are fresh

materials that are processed the same way as specimens (step D3), homogenized and stored under optimal conditions; they are aimed to be used as reference materials for optimizing existing analytical methods and/or developing new techniques, considering that the optimum representativeness of the tested materials is then achieved. LRMs in step D2 are samples that are homogenized and stabilized by freeze-drying; these materials are used as internal QC tools to monitor the method reproducibility and proficiency testing exercises among the laboratories involved in the monitoring network. Finally, specimens (step D3) are the actual stated references for producing data that will serve to follow-up the environment quality. These materials are stored under optimal conditions (specimen banking, step E) and analyzed as described in the following.

- Step E: as mentioned earlier, this is the final repository of environmental specimens that are stored over long-term periods of time.

- Step F: the box in this step corresponds to the validation of the monitoring methods. The various items have been discussed earlier, with the limitations that we may face with respect to the achievement of measurement traceability. The methods have to be validated according to the best of the state of the art, using available CRMs. The availability of IRMs and on-going testing enables progress to flow into the methods, establishing links between data produced with the methods at a given methods and possibly improved methods. This reference is hence a moving feature, but the traceability chain is preserved.

- Step G: as mentioned in step D, the validated method has to be controlled at regular intervals either by means of internal QC checks (control charts) or external QC (proficiency testing), using LRMs issued from the monitoring of reference sites.

- Step H: steps F–H are closely interlinked. The method validation (step F) enables us to define SOPs that will be followed to carry out analytical measurements on a routine basis (step H), while proceeding with regular internal and external QC checks (step G). Analytical results (specimen analyses) are obtained from the routinely operated methods.

– Step I: monitoring results are communicated to the steering committee of an international network representing regional or national entities (involved in the monitoring of reference sites at the regional or national level). The reference system has to be scrutinized by this committee and possible doubts on the traceability chain call for explanations from the monitoring laboratories. The network reports on the environment quality status on a yearly basis.

This case study is an illustration of a hypothetical structured monitoring system, with a well-defined traceability framework. The establishment of such a structure would depend upon the willingness of political decisionmakers for both initiating the creation of an international network and a possible pilot study at the international level, and ensuring long-term finance for operating this monitoring system.

5.1.4. Conclusions

Long-term (marine) environmental monitoring can only be valid if the data are obtained under a reliable QA regime [QUE 99b]. Comparability of data is mandatory for evaluating spatial and temporal contamination trends, and this is only achievable if harmonized approaches are considered, from sampling to final detection, for the monitoring of given substances in given media. Without the demonstration of data traceability to any kind of well-documented stated references, a considerable amount of data published in the scientific literature is actually totally useless and this represents a huge waste of resources. It has been questioned whether it would not be timely to establish a sound, structured, harmonized system for monitoring the environment at the planet level [QUE 04]. Fewer data of high quality in specific marine sites that would be monitored on a regular basis would certainly serve the needs for monitoring, mapping, geochemical studies, etc., much better than the wide dispersion of data being published without any information on their quality.

Monitoring the marine environment in many instances has been carried out for years following the approach shown in Figure 5.2. The

situation is improving and efforts are made internationally to establish structures to ensure comparability of data. An example of these efforts is the QUASIMEME program related to the monitoring of the marine environment [WEL 97]. We may dream of a structure as described in Figure 5.3 in which all the operations carried out in the frame of chemical marine monitoring programs would be traced back to well-defined and internationally accepted stated references. Alternatively to an “ideal” structure based on well-characterized reference sites, the long-term traceability of chemical marine measurements will only be possible if a wide array of reference materials is made available and a “chain” is established among the differently used standards. Considering that RMs are consumables, they cannot be considered the same way as transfer standards (i.e. physical standards). However, it would be of great value to store a given number of vials of each produced RM in the best storage conditions in some official organizations to maintain long-term stated references for future marine environmental measurements.

In conclusion, we should not confuse this search for traceability to well-defined and accepted stated references to the achievement of accuracy. The first concept is a moving feature, i.e. stated references may evolve with progress of knowledge and of technical capabilities, while still maintaining comparability of data, but it does not necessarily mean that the produced data are accurate (i.e. close to “true value”). This is partly compatible with the metrological principle of traceability that implies that “if the traceability of measurements is claimed to be other than the mole unit itself but rather through a procedure, material or standard, then there must be credibly described and their relation to the mole clearly established”. Indeed, the traceability of marine chemical monitoring measurements can be demonstrated to pure calibrating substances, CRMs or documented standards; the latter two often corresponding to “consensus” values and not “true” values. Demonstrating traceability of an amount of substance to its true value in a given medium is, therefore, hardly achievable in practice. We are in a world of compromises, and the best compromise to date for marine monitoring will be to achieve the best possible comparability in a system that will allow us to trace

measurement data produced over decades of analytical measurements (hence achieving a sound trend evaluation of environment quality status). This system would rely on physical tools (reference materials, specimens) and it should respond to progress in analytical sciences so that the stated references may be regularly improved, while still maintaining a traceability chain with “old data”. Indeed, if analytical progress actually enables us to refine the determination of certified (or reference) values, approaching their closeness to the true value, links with measurements carried out decades ago (but verified with RMs of lesser confidence) will still be possible and the data will not be lost. In other words, small detected biases (detected with more advanced techniques) could be corrected in the future if a system of RM banking is implemented. Therefore, we may hope that the progress in analytical chemistry will be such within the next decades that the accuracy of measurements will be firmly demonstrated, i.e. establishing traceability to the true amounts of contaminants in the environment.

5.2. Policy perspectives

Policy settings of marine chemical monitoring have undergone a considerable development over the past decades [QUE 11b]. In Europe, the adoption of the Water Framework Directive (and related directives) and of the Marine Strategy Framework Directive has provided the impulse for a reframing of the traditional marine environmental monitoring and assessment activities of the coastal EU Member States, leading in some countries to a complete reshuffling of their monitoring strategies. The overall marine environmental policy evolved from reactions to acute pollution incidents to a more comprehensive and long-term undertaking that takes a broader view of forms of chemical pollution (from prevention to response). Environmental “good status” objectives have also framed monitoring efforts to be more result driven than pre-existing strategies. Monitoring has also been broadened and conceived in consideration of more frequent assessment cycles responding to EU policy requirements (see Chapter 1). These cycles imply that temporal trend analysis be undertaken, which requires stability and dedication

of monitoring organizations. However, despite the increasing ambition of the marine environmental policy context, the day-to-day management of marine environmental monitoring may not always be conducive to such stability owing to change in priorities, resources reallocations, etc. Time series may be stopped before having delivered their “optimum” information content. This represents one of the key challenges of marine policy nowadays.

Scientific communities involved in marine chemical research and monitoring have made significant efforts to solve common methodological problems that affected their operational work, in particular statistical tools for monitoring, program design, more robust sampling methodologies and *in situ* monitoring methods taking account of technological advances (Chapter 3). Furthermore, the complexity of marine environmental matrices and the often low concentrations encountered in the different compartments provided an impulse for a wide range of innovative developments in the field of analytical chemistry (Chapter 4).

QA activities have also been of very high concern to the chemical monitoring community owing to the needs to achieve comparability of results in coastal and open marine environments. They include the use of appropriate analytical (written) standards, QA and control procedures, use of appropriate (certified) reference materials and assuring quality in data management procedures (Chapter 2 and earlier sections). As described in several places in this book, it is recognized that marine chemists have played a leading role in the development and promotion of key aspects of QA/QC and raised awareness about the need for highly competent and skilled personnel, which are a necessary condition for setting up and running successful chemical monitoring programs. These developments have resulted in a strengthened methodological base that can serve to underpin a multiplicity of monitoring programs in different marine environmental policy contexts.

Verreet [VER 11] has proposed a “SWOT” analysis (strength, weaknesses, threats, opportunities) about monitoring features,

which can be used for policies and methods described in this book:

Strengths

- Marine chemical monitoring undergoes a policy backing involving a range of legally binding instruments, including important EU directives.
- Technological advances have opened up new possibilities, in particular for the determination of emerging pollutants, and low concentrations levels of “classical” pollutants.
- A strong scientific investment has been made in the methodological basis (including a “QA culture”) for all aspects of chemical monitoring in past decades.
- The range of chemical monitoring techniques has significantly broadened.

Weaknesses

- The timing of monitoring programs often lead to delayed harvesting of results, which is due also in part to the inherent inertia of the system observed.
- The scope of chemical monitoring may be too narrowly focused on purely determining “trends” rather than on a systemic understanding of what happens to the contaminants in the marine compartments.
- Few routine monitoring programs have established strong operational links between different types of chemical, biochemical and biological effects monitoring, although this would contribute to a better understanding of the levels and effects of chemical contamination and pollution.

Opportunities

On-going intensification of marine chemical assessments at international level provides opportunities to gather as much information as possible out of the available data, demonstrating their value, and monitoring data providers can build their case on the

assessment results, and enable them to view data series over long time perspectives.

Threats

- “Chemical pollution” is often no longer a main issue for environmental managers who are instead concerned by other pressures (e.g. overfishing and habitat degradation).

- The increasing lists of emerging contaminants are not compensated by increased resources, i.e. more is expected to be done with fewer resources. Linked to this, the question of costeffectiveness and resource constraints may lead to a reduction of monitoring efforts and/or the consideration of “cheaper” methods.

- Discontinuities in data series may have long-term impacts on pollution trend studies.

- Disconnecting data providers from data users due to upscaling of database systems may lead to losing important data knowledge.

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Index

A, C, F

atomic absorption, 133, 148, 151,
152, 161, 169
contaminants
 particle-bound, 143, 145
 emerging, 191, 193, 234
 organic, 50, 59, 65, 66, 86, 124,
 132, 133, 142, 157, 193, 196
fractionation, 134, 139, 163, 165–
167

I, L, M, N

in situ methods, 95, 105
laboratory reference materials
 (LRMs), 87, 88, 161, 188, 214,
 216, 228

marine monitoring steps, 74
nutrient monitoring, 197–201

P, S, X

procedures
 QA, 74, 86, 95
 QA/QC, 40, 46
 QC, 52
proficiency testing, 22, 40, 44, 45,
 87, 136, 205, 219, 228
sampling program, 80
sample storage, 73, 83, 111, 112,
 178, 211, 221
X-ray techniques, 158, 161

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